VISUAL DISCRIMINATION PERFORMANCE IN RATS:
ROLE OF ACETYLCHOLINE AND SYNAPTIC
CORRELATES IN THE PRIMARY VISUAL CORTEX AND
HIPPOCAMPUS

By

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Abstract

The notion that learning and memory processes are highly dependent on central cholinergic neurotransmission has been widely accepted. However, studies documenting the importance of Acetylcholine (ACh) in attention have led some to suggest that attention impairments may underlie the deficits in learning and memory resulting from cholinergic disruptions. Using a visual discrimination task, I attempted to discern whether performance impairments by Scopolamine were predominantly due to the importance of muscarinic receptor integrity in attention, or memory consolidation in learning.

Rats were trained in a visual discrimination task using a Y-shaped water maze apparatus. To successfully navigate to a hidden platform located in one of the two goal arms, rats learned to discriminate between 2 distinct visual cues, indicating the platform’s presence (CS+) or absence (CS-), respectively. Following task acquisition, testing continued using a combination of Regular trials (RT; both CS+ and CS- present) and Probe trials (PT; only one of the cues present). Results indicated that performance on PT was impaired due to greater task difficulty under conditions of reduced information, while Scopolamine (1 mg/kg) further impacted PT performance without affecting RTs. In a second experiment, PTs were administered with the platform present to provide reinforcement and a learning opportunity. Animals still exhibited poorer PT performance, but rapidly learned to rely on a single cue for accurate platform localization. Interestingly, this learning was not apparent under conditions of Scopolamine treatment (1 mg/kg), even though RT performance was completely unaffected. To examine experience-dependent changes in neuronal responding after visual discrimination learning, a subset of animals were anesthetised and visual evoked potentials (VEPs) in V1 and area CA1 of the hippocampus were recorded in response to CS+, CS-, and novel stimuli. In both the V1 and CA1, the VEP amplitudes elicited to familiar and novel stimuli were not significantly different.

First, these experiments demonstrate the importance of the cholinergic system in sustaining visual attention and acquiring a new single-cue strategy. Furthermore, the null electrophysiology findings do not rule out the plastic response properties of the mature V1 and CA1, but remind us of the complex nature of memory encoding in the brain.
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<tbody>
<tr>
<td>5-CSRTT</td>
<td>5 Choice Serial Reaction Time Task</td>
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<td>ACh</td>
<td>Acetylcholine</td>
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<td>ANT</td>
<td>Attention Network Task</td>
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<tr>
<td>AP</td>
<td>Anterior-Posterior</td>
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<td>BOLD</td>
<td>Blood-oxygen-level Dependence</td>
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<td>ChAT</td>
<td>Choline acetyltransferase</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CS+</td>
<td>Positive Conditioned Stimulus</td>
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<tr>
<td>CS-</td>
<td>Negative Conditioned Stimulus</td>
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<tr>
<td>ERPs</td>
<td>Event-related Potentials</td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<tr>
<td>i.m.</td>
<td>Intramuscular</td>
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<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>i.v.</td>
<td>Intravenous</td>
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<tr>
<td>ML</td>
<td>Medial-Lateral</td>
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<tr>
<td>mPFC</td>
<td>Medial Prefrontal Cortex</td>
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<tr>
<td>LGN</td>
<td>Lateral Geniculate Nucleus</td>
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<td>LTP</td>
<td>Long-term Potentiation</td>
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<td>MS</td>
<td>Medial Septum</td>
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<tr>
<td>NT</td>
<td>Neurotransmitter</td>
</tr>
<tr>
<td>P+</td>
<td>Platform Presence</td>
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<tr>
<td>P-</td>
<td>Platform Absence</td>
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<tr>
<td>PT</td>
<td>Probe Trials</td>
</tr>
<tr>
<td>RT</td>
<td>Regular Trials</td>
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<tr>
<td>SC</td>
<td>Scopolamine</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>VD</td>
<td>Ventral-Dorsal</td>
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<tr>
<td>V1</td>
<td>Primary Visual Cortex</td>
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<tr>
<td>VDB</td>
<td>Vertical limb of the Diagonal Band</td>
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<tr>
<td>VEPs</td>
<td>Visual Evoked Potentials</td>
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Chapter 1

Introduction

In order to survive, animals must be able to quickly perceive elements in the environment using their sensory systems, process and evaluate this information, react appropriately, and store relevant experiences to regulate future behaviour. Of the various forms of sensory information available (e.g. tactile, olfactory, magnetic fields), visual cues are often used by many different species as an important guide for behaviour. As such, learning and memory tasks involving visual processing have been used by researchers to examine how novel experiences and perceptual information processing modifies the function of neural circuitry over time. Moreover, certain neurotransmitters that have been implicated in a variety of cognitive processes, such as Acetylcholine, have been studied extensively in order to elucidate one of the many underlying mechanisms of neuronal plasticity. This study examined the role of Acetylcholine in attention and the process of learning and memory, and investigated experience-dependent changes in the primary visual cortex and hippocampus to familiar and novel images after visual discrimination learning.
Chapter 2

Literature Review

2.1 The Role of Acetylcholine in Learning and Memory

Acetylcholine (ACh) is a neurotransmitter (NT) found throughout the peripheral and central nervous system (CNS), where it exhibits modulatory effects upon neural mechanisms controlling plasticity, arousal, and reward. From the 1950-60’s, a popular means of facilitating childbirth involved the administration of a relatively light dose of sedative simultaneously with a central anticholinergic drug (preventing the binding of ACh). Interestingly, doctors noticed that in addition to the altered state of consciousness termed “twilight sleep”, patients reported an inability to remember events occurring just prior to and during the procedure (Bartus, Dean, Pontecorvo, & Flicker, 1985). The impairment of recent memories associated with centrally acting anticholinergic drugs was also found in a number of animal studies (e.g., Herz, 1960; Meyers & Domino, 1964). Notably, Deutsch (1971) was the first to link ACh directly to learning and memory. From his study, he found that cholinergic synaptic conductance was altered as a result of learning, and hypothesized that sensitivity of the postsynaptic membrane to ACh increased for a period of time after initial learning. Furthermore, researchers also noted that brain areas known to be rich in cholinergic cell bodies or terminals (e.g., hippocampus, septum, frontal cortex, amygdala) also seemed to play important roles in the learning and memory phenomena (e.g., Douglas, 1967; Iverson, 1973).

In later pharmacological studies, Drachman and Leavitt (1974) reported that when given an acute dose of a central anticholinergic drug, young humans (age: 19-25) performed various tasks of a cognitive test battery in a manner similar to normal elderly
subjects. These selective drug-induced performance failures on certain memory tests hinted at a possible cholinergic deficiency in the aged brains of elderly individuals. A similar impairment in short-term memory after anticholinergic drug administration, characteristic of older animals, was also replicated in the rhesus monkey (Bartus & Johnson, 1976; Bartus, Fleming, & Johnson, 1978). At the neuronal level, Segal (1982) observed a specific decrease in responsiveness to ACh in hippocampal pyramidal cells in aged rats.

By the early 1980’s this accumulation of evidence suggested that the breakdown of the central cholinergic transmission played an important role in the earliest and primary symptoms of Alzheimer’s disease (an incurable, neurodegenerative disease characterized by an inability to acquire new memories). These results seemed to fit well with Bowen and colleagues’ (1973) reports of the significant reduction of choline acetyltransferase (enzyme joining Acteyl CoA to choline, forming ACh; ChAT) activity in Alzheimer’s patients relative to age-matched controls. Bowen et al., hypothesized that due to the widespread reduction (across various brain regions), and the degree of specificity (markers for other NT were not similarly reduced), the ChAT activity decrease could be correlated with the loss of cognitive function and increase in plaque and tangle density. They soon gained support from other laboratories that had also independently found a profound loss of ChAT activity exhibited in the brains of Alzheimer’s patients upon postmortem examination (Davies & Maloney, 1976; Perry, Perry, Blessed, & Tomlinson, 1977). Considering the implications of these findings, Bartus and colleagues (1982) proposed the Cholinergic Hypothesis, postulating that the age-related memory loss and decline in cognitive function was predominantly due to a decrease in ACh levels.
and significant functional disturbances in cholinergic activity. Since then, many researchers have supported the view that cholinergic mechanisms modulate learning and memory formation.

ACh levels have been shown to increase substantially from the first and third training days of an associative auditory task (Butt et al., 2009) in experimental but not control rats. This provides direct support for the notion of ACh release during natural memory encoding, where cholinergic activation is thought to contribute to specific associative representational plasticity occurring in conjunction with memory formation. Furthermore, pharmacological data from various studies provide evidence that both muscarinic and nicotinic ACh receptors (two major ACh receptor subtypes) have a role in encoding new memories (Hasselmo, 2006). Not surprisingly, central cholinergic antagonists for both receptor types have been shown to induce behavioural effects on different learning tasks. Traditionally, scopolamine (SC), a non-selective muscarinic receptor antagonist, has been used as a standard drug for inducing cognitive deficits in healthy humans and animals (Klinkenberg & Blokland, 2010). To address the peripheral effects of SC (e.g., pupil dilation, increased locomotor activity at high doses) that are unavoidable with standard systemic administrations (i.v., i.m., i.p.), a control group given equivalent doses of methylscopolamine is often included (Harvey, Gormezano, & Cool-Hauser, 1983). Methylscopolamine, otherwise known as scopolamine methylbromide, is a quaternary form of SC that has the same receptor binding characteristics, but does not readily cross the blood-brain barrier (Pradhan & Roth, 1968). As such, this drug enables researchers to separate the peripheral side-effects of SC from the cognitive effects brought on by central-binding activity at any given dose.
When comparing the spatial maze acquisition of control rats versus rats given SC, Stevens (1981) found that drug-treated animals failed to learn the task. However, when the same SC-treated animals were given additional training days under saline administration, they showed a marked improvement in spatial maze performance. Similarly, local infusions of SC into the parahippocampal structures have demonstrated the role of cholinergic receptors for information encoding needed for subsequent stimuli recognition in both monkeys (Tang, Mishkin, & Aigner, 1997) and rats (Winters & Bussey, 2005). In addition to SC, other pharmacological manipulations of the medial septum (major site of cholinergic neuron projections to the hippocampus) that are often associated with enhancements or impairments of cholinergic function in the hippocampus, have also been found to enhance or impair learning and memory, respectively (e.g., Farr, Uezu, Flood, & Morley, 1999; Walsh, Herzog, Ghandi, Stackman, & Wiley, 1996). Furthermore, studies documenting the effects of electrolytic and neurotoxic lesions of the medial septum have reported profound impairments in the same learning and memory tasks that are impaired by hippocampal damage alone (e.g., Decker, Radek, Majchrzak, & Anderson, 1992; Nilsson et al., 1992).

### 2.2 Acetylcholine and Attention

Despite the large body of support for the modulating effects of the cholinergic system on performance in learning and memory tasks, a vigorous debate has recently emerged regarding whether behavioral impairments are due to “learning/memory” mechanisms per se, or some other domain of cognitive functioning. Although it is argued that we have methods to control for the peripheral, cognition-independent side-effects of
cholinergic drugs, there are many stages involved in information processing and subsequent memory formation (e.g., perception, sensory processing, memory encoding and subsequent consolidation, etc.) that make it difficult to isolate the specific stage(s) that are affected by cholinergic drugs. Adding to this uncertainty are the breakthrough findings that animals lesioned with a highly specific cholinergic toxin 192 IgG-saporin (specific abolition of cholinergic basal forebrain neurons) do not show clear learning and memory impairments (e.g., Baxter & Gallagher, 1996; McGaughy, Everitt, Robbins, & Sarter, 2000). Moreover, it has been found that attention tests tend to be relatively more sensitive to treatment by SC in comparison to learning and memory tasks (Hodges, Lindner, Hogan, Jones, & Markus, 2009). In particular, SC disrupts performances in attention tasks at much lower doses before any measurable mnemonic impairment is manifested.

Apart from the widely accepted role of ACh in cognition, this NT has also been implicated in optimizing the signal-to-noise ratio in the parietal (Broussard, Karelina, Sarter, & Givens, 2009) and visual cortices (Murphy & Sillito, 1991), a feature considered vital for stimulus processing. In rats performing a sustained attention task, cholinergic disruption was found to decrease neuronal activation to detected cues, and enhance neuronal activity to distracter stimuli in neurons of the posterior parietal cortex (Broussard et al. 2009). Broussard et al. suggested that ACh played an important modulatory role in the detection and processing of relevant stimuli, and suppression of responses to irrelevant cues under attention challenging conditions. Silver, Shenhav, and D’Esposito (2008) reported that increasing ACh levels with donepezil reduced the spatial spread of excitatory responses in the human visual cortex, which they considered to be
consistent with the role of ACh in reducing excitatory receptive field size. Interestingly, it has also been found that an increase in firing rate of a V1 neuron by low doses of ACh could only be altered with a muscarinic antagonist SC, but not a nicotinic antagonist mecamylamine (Herrerro et al., 2008), thus underlining the importance of muscarinic but not nicotinic receptor mechanisms in cholinergic attentional modulation.

Cortical cholinergic activity has also been linked to the novelty of the stimulus (Miranda, Ramirez-Lugo, & Bermudez-Rattoni, 2000). When evaluating ACh levels in the insular cortex during presentations of different taste stimuli, researchers found that ACh release after several presentations of a previously novel taste, decreased to the same levels as those produced by a familiar taste. These results indicate an inverse relationship between familiarity and cortical ACh release, which suggest that the cholinergic system is important for identifying and characterizing different stimuli. In many situations, attentional mechanisms are involved in identifying and characterizing stimuli. Therefore, not surprisingly, damage to the frontal lobe – an area thought to be important for directing visual attention, and which incidentally is high in ChAT activity (Javoy-Agid et al., 1989), has been found to impair one’s ability to respond normally to novel events (Daffner et al., 2000).

Taking into account these recent developments illustrating the importance of ACh in attention, it has been proposed that the traditional view of ACh involvement in learning and memory should be reevaluated. Some researchers have suggested that the impairing effects of anticholinergic drugs (e.g., SC) on performance in learning and memory tasks should instead be interpreted in terms of the role of ACh in attention and sensory/stimulus discriminations (Klinkenberg & Blokland, 2010). Whether ACh
influences the actual encoding and memory consolidation process, or the initial sensory processing stages and attention remains controversial. As such, future studies, such as the one proposed in this thesis, must find a way to properly separate and assess the effects of ACh on these processes in order to better characterize its role.

2.3 Adult Visual Cortex Plasticity

Contrary to earlier ideas that memory is a specialized property of a few cortical areas, researchers now believe that all areas of the cortex are capable of experience-dependent changes over various time scales (Gilbert, Sigman, & Crist, 2001). While sensory cortices, such as the primary visual cortex (V1) were initially assumed to be relatively stable and “hardwired”, recent evidence suggests that even these early sensory modules are capable of plastic reorganization (e.g., Tsanov & Manahan-Vaughan, 2008). The notion of a fairly stable V1 structure likely stemmed from a suggestion made by Hubel and Wiesel (1970) that the visual cortex was not susceptible to changes induced by visual deprivation once the critical period of development had ended in young animals. Although certain functional properties of the visual system, such as ocular dominance, generally remain relatively fixed after a critical period in postnatal development (Hubel & Wiesel, 1977), recent findings support the occurrence of experience-induced synaptic plasticity in the adult V1 (Heynen & Bear, 2001).

Perceptual learning, or the specific and relatively permanent modification of perception and behaviour following sensory experiences (Fahle & Poggio, 2002), has been observed in a variety of tasks that involve the visual system (e.g. visual acuity). In such tasks, the neural site of modification is inferred by the pattern of learning transfer
More specifically, if the learning is assumed to occur in early visual circuitry (i.e., V1), where neurons are orientation-selective and have small receptive fields, the learning effects will be specific to the attributes of the stimulus such as orientation (Ahissar & Hochstein, 1997). That is, training at one visual field position will only trigger a change in neural activity corresponding to a set group of neurons, with others remaining unaffected. However, if differences in neuronal activity are present in untrained areas, plasticity is likely occurring higher up in the visual processing hierarchy (Hochstein & Ahissar, 2002), where neurons have larger receptive fields and are less sensitive to particular stimulus attributes. From recent studies, a growing body of evidence seems to support the hypothesis that many forms of perceptual learning occur at early cortical processing stages of visual information (Tsanov & Manahan-Vaughan, 2008).

In a study conducted by Furmanski, Schluppeck, and Engel (2004), visual learning was shown to selectively increase the overall response to trained stimuli in the human V1. By comparing the neural signals measured by functional magnetic resonance imaging (fMRI) before and after one month of practice at detecting extremely low-contrast oriented patterns, researchers witnessed a 39% increase in V1 response for practiced orientations relative to control orientations. Similar changes in signals localized to V1 were also found after participants extensively practiced a more complex task involving curvature discrimination on illusory contours (Maertens & Pollmann, 2005). As with the previous experiment, the clear increase in blood-oxygen-level dependence (BOLD) responses to illusory contours was observed in V1 from pre-training to post-training. Perhaps even more interesting was the high level of specificity of the signal...
increase observed in V1. Not only was there a retinotopically specific BOLD response enhancement to the illusory contours used in training, but this amplification was only found in subjects who had improved significantly in the discrimination task over the course of the experiment. Furthermore, the increased neural activity resulting from perceptual training appeared to be long-term, as it was maintained over a period of 10 months in a manner specific to the trained visual field position.

Although these recent findings have helped to localize the visual areas involved in perceptual learning, isolating the mechanisms responsible for the change at the cellular level remains a challenge. Some have proposed that the neurons involved in processing the trained stimulus become potentiated, and therefore increase their response magnitude to particular cues (i.e. greater firing). Alternatively, there might also be a recruitment of additional cells during learning which may lead to a greater number of neurons representing the learned stimulus – a hypothesis that has been supported by evidence obtained in other sensory modalities (e.g., auditory, somatosensory; Recanzone, Schreiner, & Merzenich, 1993; Recanzone, Merzenich, Jenkins, Grajski, & Dinse, 1992).

In an attempt to directly study the physiological correlates of perceptual learning effects at the level of individual neurons, Ghose, Yang, and Maunsell (2002) trained monkeys in an orientation discrimination task and subsequently took electrophysiological, extracellular recordings of single neurons in V1 and V2. During the task, all behaviorally relevant stimuli were restricted to a particular retinal location, and were moved about in a specific orientation. Despite the fact that monkeys exhibited a gradual improvement in discrimination thresholds specific to the trained orientation, this learning caused little change in response patterns of V1 and V2 neurons. From this, it was
concluded that behavioural improvement was accomplished without altering the signal responses from early visual neurons.

A possible reason for this somewhat unexpected finding might be that the task in the Ghose experiment was too simple to cause a change in neuronal activity, as it has been previously shown that the difficulty of a visual task can affect the extent of V1 involvement in learning (Ahissar & Hochstein, 1997). The findings by Crist, Li, and Gilbert (2001) illustrated the importance of taking into account the context in which a stimulus is presented and recorded. After training macaque monkeys to perform a perceptual discrimination task, these researchers found that receptive field properties and visual topography were indistinguishable between trained and untrained animals. Remarkably however, the addition of contextual stimuli placed outside the receptive field during the task triggered an enhanced response to trained stimuli. These results indicate that training-dependent changes in receptive field properties may emerge only under the appropriate (and often complex) viewing conditions of the experimental subject.

2.4 Plasticity in the Visual Cortex of Adult Rodents

While the neural substrates associated with non-human primate perceptual learning seem to be task-reliant, the relationship between perceptual experience and visual cortical changes in rodents thus far appear to be more straightforward (Karmarkar & Dan, 2006). Frenkel and colleagues (2006) found that the repeated exposure of awake-mice to certain visual stimuli (dark bars of specific orientations) alone was sufficient to induce specific potentiated responses in V1 to the trained orientations. Interestingly, this improvement was isolated to the trained eye and took place regardless if the mice were
adults or juveniles. Although the development of such an effect only occurred across multiple days of training, these results were still unexpected due to the passive nature in which it was acquired (animals were not required to actively perform any task). In addition to visual evoked changes in neural responses, V1 neurons have also been shown to develop sensitivities to non-visual stimuli paired with visual cues in a learning task (Shuler & Bear, 2006). In a paradigm where freely-moving rats were taught to pair different reward times with visual stimuli available to both eyes, Shuler and Bear discovered that a significant proportion of visual cortical neurons showed firing patterns that correlated with the expected reward time.

More recently, Hager and Dringenberg (2010) examined whether neurons in the visual cortex of rats would change in their responsiveness to visual cues if they were trained in a visual discrimination task. Rats trained in a water Y-maze task learned to discriminate between two visual cues that indicated the presence (P+) or absence (P-), respectively, of the hidden escape platform. Control rats received the same procedure, but visual cues did not have a predictive relationship to platform location. After the task was learned, task-trained, control, and naïve rats were anesthetized to allow recordings of visual evoked potentials (VEPs) in V1 to the presentation of P+, P-, and a novel visual cue (not encountered during training). Results indicated that animals that had learned the discrimination task showed higher amplitude VEPs to cues encountered during training (P+/P-) in comparison to the novel cue. In naïve and control animals, similar VEP amplitudes to the different cues indicated a clear shift in visual cortex response as a result of discrimination learning.
From the primate and rodent research presented here, perceptual learning in the visual system appears to be mediated mostly by modifications in response-strength of individual neurons, rather than the large-scale spatial reorganizations of the cortex found in the auditory and somatosensory cortices (Karmarkar & Dan, 2006). Perhaps future research will help determine whether these differences are attributable to the qualities of the perceptual training paradigms used, or to the specific characteristics of the visual cortex circuitry. At the present time, there is clear evidence for experience-dependent synaptic plasticity in V1 that researchers propose may be involved in influencing subsequent stages of information processing in long-term memory formation (Tsanov & Manahan-Vaughan, 2008). In particular, V1 plasticity is thought to influence the ability of the hippocampus to integrate spatial episodes in time (Ji & Wilson, 2007). As such, this interaction may be a key component in the neocortico-hippocampal transfer of information underlying the formation of spatial memories.

2.5 Hippocampal Involvement in Visual Discrimination Tasks

The hippocampus is an evolutionary older structure of the limbic system that plays an important role in the consolidation of specific information from short-term to long-term memory, as well as spatial navigation. Many experiments have shown that the hippocampus is part of the critical circuitry mediating storage of spatial information used to guide navigational behavior in rats and other species (e.g., O’Keefe & Dostrovsky, 1971; Jarrard, 1995; Moser & Moser, 1998; Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002). Despite this evidence, some studies have suggested that simple sensory discrimination abilities and memories are not dependent on hippocampal
integrity. For example, a number of experiments have confirmed that hippocampal damage does not affect *simple* discrimination learning (Wishaw & Tomie, 1991; Alvarado & Rudy, 1995). In this type of learning, the subject is trained, through many trials, to associate one of the two available stimuli with reinforcement. From the lack of learning deficits observed after hippocampal damage, many have concluded that the hippocampus is not necessarily needed for simple discrimination learning. However, the degree of hippocampal formation involvement in this type of learning may be, at least in part, dependent on the specific cues (and associated sensory modalities) employed for the discrimination procedure. For example, hippocampal-damaged rats were found to perform at control levels when assessed in an olfactory or two-object discrimination task (Broadbent, Squire, & Clark, 2007). However, when rats with hippocampal damage were compared to controls in their ability to acquire and retain a visually-based, 2-pattern discrimination task, results no longer suggested a total lack of hippocampal involvement. Despite finding no significant differences in the acquisition-rate of the task, it was noted that 3 out of the 9 hippocampal-lesioned animals never learned the task and were excluded from the data analysis. Moreover, even among the lesioned rats that acquired the task, a test administered 2 weeks after the training revealed a marked impairment of long-term retention in comparison to controls. Perhaps in most cases, simple discrimination tasks are independent of hippocampal processing (e.g., odor); however, it is possible that when a visual component (e.g., pattern discrimination) is added, this no longer holds true.

The results from Epp and colleagues (2008) present yet another aspect to the debate of whether the hippocampus is needed for discrimination learning – in particular,
visual discriminations. According to Epp et al. (2008), rats readily learned simple two-choice picture discriminations even after being subjected to hippocampal damage – a finding not necessarily consistent with that of Broadbent et al. described above. However, if such discriminations were learned before the operation (hippocampus still intact), later damage caused severe retrograde amnesia for these discriminations instead of the anterograde amnesia-like effects observed by Broadbent et al. Furthermore, retrograde amnesia for previously learned cues was equally severe regardless of whether the interval between training and hippocampal damage was 1 or 60 days. Researchers also found that the severity of retrograde amnesia for simple picture discriminations was correlated with the extent of hippocampus damage. Thus, these authors concluded that although simple discrimination learning could proceed in the absence of the hippocampus, this area normally supported this type of learning by storing long-term memories for visual information encountered during task acquisition. Together, the findings from these two studies suggest that, when intact, the hippocampus will take part in the long-term retention of memories during discrimination learning that involve visual information.

2.6 Influences of Visual Processing on Hippocampal Plasticity

Now we return to an idea presented earlier; if one accepts that the hippocampal formation uses visual information, which has elicited some form of plastic reorganization in the ventral stream of visual processing, it is then logical to hypothesize that these synaptic alterations can also evoke plastic responses in the hippocampus (Tsanov & Manahan-Vaughan, 2008). In primates, the ventral pathway is a set of cortical areas spanning from the early visual cortex areas to the inferior temporal cortex, carrying
information from the retina to the hippocampus. Upon reaching the inferior temporal cortex, visual information continues via the perirhinal and entorhinal cortices, which finally project onto the hippocampus. The recent literature appears to be consistent with the notion of an interaction of visual and hippocampal plasticity. By taking parallel field recordings from the visual cortex and dentate gyrus of the hippocampus, Tsanov and Manahan-Vaughan (2006) revealed an interaction between synaptic alterations in both structures. Long-term potentiation (LTP) is a mechanism of plasticity that is defined as a relatively stable enhancement of synaptic transmission between neurons following synchronized electrical stimulation (Bliss & Collingridge, 1993). One way to induce LTP in a brain area electrically is to employ theta-burst stimulations. After theta-burst stimulation of the thalamocortical pathway, Tsanov and Manahan-Vaughan found a lasting enhancement of granule cell excitability in the hippocampus that was accompanied by a concurrent V1 response potentiation. Noticing the correlation between the evoked potentiation in V1 with the enhancement of intrinsic dentate gyrus excitability, the authors suggested that this may facilitate the induction of synaptic plasticity in the hippocampus.

In support of this idea, other evidence suggests that neocortical cellular excitation may effectively drive and induce plasticity (and long-term storage) of information at hippocampal synapses (Ji and Wilson, 2007). Ji and Wilson (2007) noted that neuronal activity patterns present during waking behaviour were replayed during sleep in both the visual cortex and the hippocampus. Not only was the replay of events in both areas coordinated to reflect the same experience, the fact that cortical activity preceded hippocampal activity by 50 ms indicated that observed hippocampal response could be a
result of cortical drive. Thus, one of the many functions of sleep may be to reprocess new information or sensory experiences encountered during waking hours, and consolidate them into long-term memories (Stickgold, Hobson, Fosse, & Fosse, 2001). In the future, to examine the influence of neocortical excitation on hippocampal plasticity further, it would be worthwhile to simultaneously record neuronal responses in V1 and hippocampus elicited by visual stimuli used previously in a learning and memory task.

2.7 The Present Study

In this study, I hope to address two major objectives through the use of behavioural, pharmacological, and electrophysiological techniques. First, given the recent controversy surrounding how the role of Acetylcholine in learning and memory should be interpreted, I will examine the role of ACh in visual discrimination learning using a modified water maze procedure. Through daily training (10 Regular trials per day), each rat will learn to discriminate between two distinct visual cues mounted on each of the two goal arms of a Y-shaped water maze insert (see Figure 1 in Methods). As such, rats will learn to associate one visual cue (CS+) with the presence of a hidden escape platform, and the other visual cue (CS-) with platform absence. Although previous work has shown that rats are capable of discriminating between different visual cues to locate a hidden platform, the role of ACh in the performance of this task has never been examined. Thus, I hope to use a modified version of the visual discrimination paradigm to evaluate the importance of ACh muscarinic receptor integrity in: 1) visual attention, and 2) memory encoding and consolidation. In order to accomplish this, rat performance on the visual discrimination task will be evaluated for an additional 5 days after acquisition (8/10
correct trials, 3 consecutive days). During this period of post-acquisition testing, rats will still be given a total of 10 trials each day, but these trials will consist of 8 Regular trials (RT; both visual cues present) and 2 Probe trials (P; only 1 of the 2 visual cues present, CS+ present for one Probe trial and CS- present for the other). For both Experiments I and II, Regular trial performance will be used as a within-subjects control for the peripheral side-effects of the drugs employed during testing.

During post-acquisition testing in Experiment I, the platform will be removed for Probe trials only, in an effort to prevent reinforcement for correct performance. By limiting the opportunity for learning a further strategy to solve single-cue guided discriminations, I argue that performance on Probe trials provide a way to measure (to some degree) the visual attention involved in a slightly more challenging situation (less visual information). For each post-acquisition day, experimental animals will be given a different dose of the anticholinergic drug SC (5 different doses: either Ascending order – 0 (saline), 0.125, 0.25, 0.5, 1.0 mg/kg SC, OR Descending order – 1.0, 0.5, 0.25, 0.125, 0 (saline) mg/kg SC) 30 minutes before testing, whereas control animals will receive no injections. First, by comparing Probe and Regular trial performance in No Injection controls, I will be able to assess whether attentional demands are in fact greater when less information is available, and whether reliance on the positive (CS+) or negative (CS-) cues differ. I hypothesize that under an injection-free condition, rats will perform significantly worse on Probe trials versus Regular trials because the task is more challenging when less visual information is present. Furthermore, due to the fact that the positive cue is paired with the platform (i.e., reinforcement), I expect to see more mistakes committed when the CS+ is removed, indicating a stronger reliance on the
positive cue. Secondly, the drug regiment employed for the experimental rats will enable me to observe SC effects, at different dosages, on performance in trials where visual information is reduced. Therefore, I hope to use Probe trial performance to investigate the importance of the cholinergic system in visual attention when less visual information is available and the task becomes more challenging. Finally, similar to the No Injection controls, I am interested to see whether a reduction in available ACh muscarinic receptors will impact cue reliance. I hypothesize that if ACh muscarinic receptors are important for visual attention, animals will exhibit a dose-dependent decrease in Probe trial performance with increasing SC doses. With regards to cue reliance, I hypothesize that at lower doses of SC, animals will rely more on the CS+, but due to the attention-impairing effects of SC at higher doses, animals will perform poorly regardless of which visual cue is presented during a Probe trial.

Throughout post-acquisition testing in Experiment II, the platform will be present during Probe trials to provide reinforcement for correct decisions. By allowing platform presence to be associated with correct Probe trial performance, rats will be given an opportunity to acquire a new strategy to solve the task when only a single cue is presented. Control rats will receive no injections, whereas the experimental animals will receive daily injections of 1.0 mg/kg SC, 30 minutes before testing. It is hypothesized that if ACh modulates memory encoding and consolidation (therefore the subsequent acquisition of new strategies), animals receiving SC will perform significantly poorer on Probe trials in comparison to control animals, despite platform reinforcement. However, animals injected with SC will continue to perform like control animals on Regular trials.
because ACh muscarinic receptor blockade should not interfere with a strategy that has already been learned and acquired.

Given the wealth of research that has surfaced in the last decade alone, the intriguing relationship between experience-dependent changes in the primary visual areas and the traditional consolidation powerhouse – the hippocampus, is an avenue that must be explored further. As such, my second main objective is to continue to examine experience-dependent changes in visual cortex and hippocampus following extensive visual discrimination training. First, I hope to replicate the visual response enhancement elicited by familiar visual stimuli (visual cues used in training) that has been measured previously in V1 (Hager & Dringenberg, 2010). Moreover, I wish to provide evidence for a similar augmentation of visually evoked potentials in the CA1 of the hippocampus that may lend further support to the modulation of hippocampal plasticity by early visual processing changes. In order to accomplish this, a subset of animals from Experiment I and II will undergo further electrophysiological procedures after the additional 5 days of post-acquisition testing are completed. Under deep urethane anesthesia, visual evoked potentials (VEPs) will be simultaneously recorded in V1 and CA1 of all animals in response to different visual stimuli (familiar: CS+ or CS-, and novel) displayed. It is hypothesized that in both V1 and CA1, greater VEPs will be measured in response to familiar (CS+ and CS-) versus novel visual stimuli presentations.
Chapter 3
Methods

3.1 Subjects

Experimental procedures were conducted on adult male Long-Evans rats (300-500g, Charles River, Quebec) in accordance with the published guidelines of the Canadian Council Of Animal Care (CCAC) with approval granted by the Queen’s University Animal Care Committee (QUACC). Subjects were housed in pairs within polycarbonate cages equipped with PVC tubing in the main colony room (12:12-h reversed light cycle) with free access to food and water. All experimental procedures were carried out during the dark phase of the light-dark cycle (7am-7pm).

3.2 Y-maze Visual Discrimination Apparatus

The visual discrimination training took place in a Y-maze inserted in a modified water maze (Figure 1). The water maze was a circular pool (180 cm in diameter, 60 cm in height) made of white Perspex, and was filled with water (~40cm deep; maintained at 22 +/- 2°C) that had been mixed with copious amounts of non-toxic white paint to achieve an opaque consistency. The Y-maze insert (height: 61 cm, length: 140 cm, width narrow end: 51 & wide end: 81 cm) was constructed of clear plexi-glass, with the two goal arms separated by a 50 cm black plexi-glass divider. During experimental trials, a rectangular platform (height: 38 cm, width: 12 cm) made of clear plexi-glass was submerged 2 cm below the water surface within the Y-maze, roughly 5 cm from the end of the goal arm. Due to the milky quality of the water, the platform was not visible at any time, and its location remained hidden throughout the experiment.
Figure 1. **Y-maze apparatus Within a Circular Water Maze**

The Y-maze apparatus that was used for visual discrimination training and its dimensions are shown. White paint was added to the water used to fill the water maze (depth of ~40 cm) to maintain the level of opacity shown above.

### 3.3 Visual Cues

Three distinct geometric patterns on laminated pieces of paper (21.6x28 cm) were adapted from Hager and Dringenberg (2010) and used as visual cues during discrimination training. The cues were as follows (*Figure 2*): 3 solid black vertical bars (2.9x14.5 cm) placed 2.9 cm apart, 3 horizontal black bars (14.5x2.9 cm) also placed 2.9 cm apart, and a solid black diamond (14.5x14.5cm) placed at the center of the page covering the same surface area of the other two designs. Two of the three visual cues were randomly assigned to each animal and mounted on the end walls of the two goal arms, just above the surface of the water (*see Figure 1*). During training, animals learned to discriminate between the two cues based on their respective association with the hidden platform. The picture indicating the presence of the platform was referred to as the Positive conditioned stimulus (CS+), whereas the picture indicating the absence of the
platform was denoted the Negative conditioned stimulus (CS-). Finally, the cue that the rat was not shown during training, and therefore was unfamiliar with, was referred to as the Novel stimulus (novel). To control for any possible biases, the usage of all cues and their designation as the CS+, CS-, or novel was counterbalanced, thus ensuring that each cue had an equal opportunity of being presented positively, negatively or not at all.

![Figure 2. Three Distinct Visual Cues used in Visual Discrimination Training](image)

The images above were adapted from Hager and Dringenberg (2010) and used throughout the visual discrimination task. For each animal, one of the visual cues served as the CS+ (platform presence), CS- (Platform absence), and Novel (unfamiliar; the usage of all cues was counterbalanced).

### 3.4 Visual Discrimination Training

The task acquisition training was divided into 3 phases: Habituation, Visual Discrimination Training: Stationary Platform, and Visual Discrimination Training: Moving Platform. For each trial throughout all phases, animals were released into the pool facing the wall, with their back turned against the goal arms. During each trial, the discrimination performed by the rat was judged and recorded as correct or incorrect. A rat’s “choice” of a goal arm was defined as the first arm in which 50% of its body entered an arm past the black divider. Therefore, in order for a choice to be deemed correct, the only goal arm that the rat “chose” must have been the one in which the platform was hidden. During breaks in training, animals were kept temporarily in a holding cage
(45x24x20 cm) with a modified base designed to allow for water drainage. Upon the completion of training each day, animals were towel dried and left under a heat lamp for 10-15 minutes before they were returned to their respective home cages.

3.4.1 Phase 1 – Habituation

In the absence of any visual cues, the habituation day was designed to introduce the animal to the testing environment and to familiarize them with swimming inside the apparatus to find the hidden platform. Each animal received a maximum of 20 trials, separated into 2 blocks of 10 trials each. To begin each trial, the rat was released facing the maze wall and was allowed 60 s of swimming to find the platform. If 60 s elapsed without the successful localization of the platform, the experimenter manually guided the rat to it. Once the rat mounted the platform, they remained there for 10-15 s until the start of the next trial. Rats proceeded to repeat this procedure until they achieved 5 consecutive correct responses, or completed a maximum of 10 trials; depending on whichever one occurred first. After this trial block, animals were placed into the temporary holding cage and allowed a 5 min rest period before the second trial block commenced. During the first block, the platform remained in the same position for all 10 trials. However, the platform was moved to the opposite goal arm for the second block, and remained hidden in that goal arm for the next 10 trials.

3.4.2 Phase 2 – Visual Discrimination Training: Stationary Platform

On this day, the same training procedure used previously in the habituation phase was employed again with the addition of two visual cues (CS+ and CS-) to the apparatus. Each animal was randomly assigned to a set of cues: the CS+ image was always mounted
on the wall of the goal arm baited with the escape platform (remain fixed throughout the trial block), whereas the CS-image was mounted on the wall of the other goal arm (paired with platform absence). Once one trial block was complete, the location of the platform was switched to the previously unbaited arm, and the visual cues were moved accordingly.

3.4.3 Phase 3 – Visual Discrimination Training: Moving Platform

This phase of training commenced on the day immediately following the nonrandom acquisition procedure, and continued until the animal reached a preset criterion (outlined below). Each day, all animals completed a total of 10 trials with 30 s rest intervals in the holding cage after each trial. As the name implied, the platform location was changed in a randomized fashion for each trial, according to a sequence produced by an online number generator (e.g. 1 = left, 2 = right). Using the two visual cues as a guide, the rats eventually learned the information provided by the images and swam directly to the arm holding the escape platform. At the end of each day, the number of correct trials were tallied and compared to the performance on the previous day. Criterion was reached once the animal achieved at least 8/10 trials correct for three consecutive days. An animal was considered to have acquired the task upon reaching criterion, and as such, subsequent training ceased. Any animal that did not reach the criterion by the 20th day of Phase 3 training was excluded from the study.
3.5 Post-acquisition Testing

Up until this point, all animals had undergone the same visual discrimination training, but after reaching criterion, the subsequent testing procedure depended both on the experiment, and group that the animals had been randomly assigned to. In order to minimize confusion, I have outlined the follow-up testing procedure for each experiment separately (below) before describing the final electrophysiological component.

3.5.2 Experiment I

Upon reaching criterion, animals were randomly assigned to either the ascending or descending scopolamine dose conditions (n=12) or the No Injection control group (n=6). The ascending and descending SC dose procedure was useful for controlling for any between-subject effects and minimizing the amount of animals needed for testing. For the ascending dose condition, rats received an injection of saline (injection control) on the first day, and incrementally higher doses of scopolamine for each of the four subsequent testing days (0.125, 0.25, 0.5, 1.0 mg/kg). In the descending condition, the rats began with the highest dose of scopolamine (1.0 mg/kg) and worked backwards, receiving a lower dose each day until they finished with the saline injection on the last day. All injections were administered intraperitoneally (i.p.) 30 minutes before visual discrimination testing each day. After the 30 minutes had elapsed, rats were placed in the Y-maze apparatus and underwent a procedure similar to their regular training days. However, the 10 discrimination trials were comprised of 8 Regular trials (RT: both CS+ and CS- present) that were identical to those during training, interspersed with 2 randomly positioned Probe (PT) trials (e.g.: RT, RT, RT, PT, RT, RT, RT, RT, PT, RT). Although the placing of Probe trials among the RTs varied, they were not presented back-
to-back, nor did they serve as the first or last trials. During Probe trials, either the CS+ cue or the CS- was removed so that only one cue remained mounted in one of the goal arms. To prevent the animals from receiving any platform reinforcement during Probe trial testing, the escape platform during Probe trials was removed from the maze and the rats were manually removed from the maze before they reached the end of the chosen goal arm. For each of the 5 post-acquisition days, the experimenter continued to record rats on their performance on both the Regular and Probe trials. Once the five days of additional testing were completed, No Injection control rats progressed to the final surgical portion of the experiment (see the Electrophysiology methods below).

3.5.3 Experiment II

To examine whether or not any additional learning took place when the rats were required to make a decision in the presence of a single cue only (reduced information), animals were randomly assigned to one of two conditions: No Injection (control; n=12), or Scopolamine (experimental; n=12). For each of the 5 days of post-acquisition testing, rats either received no injections (controls), or 1.0 mg/kg of Scopolamine, 30 minutes before daily testing (depending on their condition). Rats were marked on their performance in 10 discrimination trials consisting of 8 RTs, and 2 PTs. However, during the Probe trials, the platform was left in the goal arm in order to provide decision reinforcement (thus allowing for acquisition of the new strategy for reduced information, if it were to occur). On the day immediately following the 5th day of testing, No Injection control animals (n=12) underwent surgical procedures (see Electrophysiology methods below). Given that rats also received regular trials (both CS+ and CS- present) on each test day, we decided to use the performance on these trials as a within-subjects control
procedure for the peripheral motor and sensory effects of the injection of scopolamine. In other words, if Probe trial performance was impaired because of the motor or sensory effects of Scopolamine, performance on the Regular trials should have also been negatively affected. By using the Regular trial performance as a within-subjects control, I was able to omit an additional group of methyl-scopolamine (does not cross blood-brain-barrier) injected animals that would have controlled for the peripheral effects of Scopolamine.

3.6 Electrophysiology
3.6.1 Surgical Preparation

On the morning following the last day of testing, electrophysiological procedures were carried out under deep Urethane anesthesia (1.5 g/kg administered i.p.) on No Injection control animals from Experiment I (n=6) and II (n=12), and a subset of rats from the Pilot Experiment (n=14). Animals from the Pilot Experiment received the same acquisition training and post-acquisition procedures as the Experimental rats from Experiment I (i.e., ascending or descending doses of SC), with the exception that the escape platform remained within the maze during Probe trials. To place animals at surgical plane, all rats were weighed and given an initial dose of 1.0 mL of urethane, and after a 30 min period, two 0.5 mL doses separated by 20 minutes were administered. Urethane supplements (~0.5g/kg) given prior to the onset of data collection was sufficient to ensure that spontaneous high frequency, low-amplitude activation on the electrocorticogram did not occur during the experiment. When breathing shifted to the lower abdominal area, and reflexes in response to hard toe and paw pinching tests were absent, the rats were given an intradermal (2 mg/kg) dose of Marcaine (local anesthesia).
Rats were then placed into the stereotaxic apparatus, and their body temperature was maintained between 36-37 °C through the use of blankets and heating pads. After a longitudinal incision was made, the skull was exposed and small skull holes were drilled into the right hemisphere to allow access to the V1 (anterior-posterior (AP) -7.5 mm, medial-lateral (ML) +3.5 mm, ventral-dorsal (VD) -.5 to -1.0 mm) and CA1 (AP -4.16 mm, ML +3.0 mm, VD -3.0 mm). To make room for the ground and reference screws, a hole was drilled over the prefrontal and cerebellum areas, respectively, in the left hemisphere.

3.6.2 Visual Evoked Potential Recordings

After both the V1 and CA1 electrodes were lowered and placed appropriately for optimal recording (Figure 3), the right eye was closed and stimuli was presented to the animals. A flat-screen computer monitor was positioned 55 cm from the animal and Direct RT Precision Timing Software (version 2004.3.0.27) was used to present the stimuli and trigger recording. The stimuli presented were bitmap images (resolution: 621 x 480) with the same dimensions as the cues presented during visual discrimination training. To survey the response in the two areas, an initial test was conducted using 16 stimuli (a repetition of CS+, CS- and novel) displays, with each image presentation lasting 5000 ms in duration. Following this sample, this initial test was repeated with a barrier placed between the monitor and animal to block the visual processing of the cues. This control procedure was used to verify that the responses recorded in the CA1 and V1 were in fact evoked by the visual input, and not by the potential electrical and magnetic disturbances present. After this had been determined, 300 stimuli (5000 ms each in
duration) presentations were displayed to each rat (100 times for each visual stimulus) with the monitor directly in front of its head (Figure 4A). The visual evoked potentials (VEPs), measured as local field potentials in response to visual stimuli recorded simultaneously in the V1 and CA1, were differentially recorded (Teflon insulated stainless steel wire, 125 -µm tip diameter) against a ground screw placed in the area of skull above the cerebellum. After this was complete, the monitor was moved to the left side of the animal, directly in line with the left eye (Figure 4B) and the 300 stimuli presentations were again displayed. In total, all rats received 300 stimuli presentations from both the front and side positions (600 in total).

At the end of each experiment, the final depths for the electrodes were noted, and rats were given a top-up injection of urethane and taken out of the stereotaxic apparatus. After perfusing through the heart with 10% formalin, the brain was removed, and standard histological techniques were employed in order to verify all electrode placements. Data obtained with inaccurate placements were excluded from the data analyses.

![Figure 3. Electrode Placements in the Visual Cortex (V1) and Hippocampus (CA1)](image)

A schematic representation of the recording electrode placements in the (A) Primary visual cortex (anterior-posterior (AP) -7.5 mm, medial-lateral (ML) +3.5 mm, ventral-dorsal (VD) -.5 to -1.0 mm) and (B) Hippocampus (AP -4.16 mm, ML +3.0 mm, VD -3.0 mm). Diagrams are adapted from the rat brain atlas by Paxinos and Watson (1997).
Figure 4. Monitor Placement for Visual Stimuli Presentations

For 300 visual stimuli presentations (100 presentations each of the CS+, CS-, and Novel images) the flat screen monitor was placed directly in front of the head of the rat (A), and for the next 300 visual stimuli presentations, the monitor was placed to the side of the animal, directly in line with the opened left eye (B). The rat’s head was held in place within the stereotaxic apparatus by the two ear bars shown in the images above.

3.7 Data Analysis

Visual discrimination performance data, measured in percent (%) correct (number of correct trials per day divided by the total number of trials per day), was averaged across animals and expressed as mean ± standard error of the mean (SEM). All statistical analyses were conducted using SPSS (version 18.0 from the SPSS Inc., Headquarters Chicago, IL) and Microsoft Excel 2007.
3.7.1 Acquisition

First, a one-way analysis of variance (ANOVA) was conducted in order to evaluate whether the number of days to reach performance criterion was affected by the particular visual cue set used for the discrimination (i.e., Horizontal and Vertical, vs. Diamond and Horizontal, vs. Diamond and Vertical). Next, for both experiments, an initial repeated-measures ANOVA (within-subjects factor: Training day) was computed to determine whether changes in performance across the first 10 days of training was indicative of learning. Although rats were not separated into their post-acquisition experimental conditions during acquisition, a mixed-model ANOVA was used to compare acquisition performance (prior to any pharmacological procedures) between post-acquisition conditions to detect any pre-existing performance differences.

3.7.2 Experiment I – Post-acquisition Testing

For No Injection control animals, a repeated-measures ANOVA was used to compare performance between trial types (Regular – 2 visual cues present vs. Probe – only 1 visual cue present) across 5 days of post-acquisition testing under a drug-free condition. For the animals that received saline and varying doses of SC (0.125 – 1.0 mg/kg), a mixed-model ANOVA (between-subjects factor: Dose Order – ascending or descending, within-subjects factors: Drug Dose, Trial type) was first used to assess whether the ascending vs. descending drug regiments affected performance differentially. If dose order did not significantly affect visual discrimination performance, data from the ascending and descending subgroups were collapsed and Probe and Regular trial performance in animals was compared after an injection of saline or varying dosages of
SC (0.125 - 1.0 mg/kg). However, if a significant main effect of dose order was found, the initial mixed-model ANOVA was used to compare Probe and Regular trial performance after varying doses of SC. For all significant main effects found using the ANOVAs, appropriate followed up post-hoc tests were computed. Finally, using Probe trial data, two separate Chi-Square tests (one for each group) were performed to examine whether reliance on the positive or negative cue (CS+ and CS-) deviated from expected values (equal reliance on both cues): a) across 5 post-acquisition days in the absence of injections, b) at different doses of SC.

3.7.3 Experiment II – Post Acquisition Testing

A mixed-model ANOVA (between subjects factor: Post-acquisition Drug condition – No injection and Scopolamine, within-subjects factors: Trial type, and Post-acquisition day) was used to compare Probe and Regular trial performance among injection-free controls and animals given 1.0 mg/kg of SC across 5 days of post-acquisition testing. In order to further investigate significant interactions, specific error terms taken from the mixed-model ANOVA output were used to perform follow-up mixed model t-tests in Microsoft Excel.

3.7.4 Electrophysiology

Using the analyze function of the Scope software offline (version 3.6.5, ADInstruments, Inc., Colorado Springs, CO), the visual evoked potentials (VEPs) recorded from the V1 and CA1 from both the front and side positions for each animal were examined (i.e., 300 VEPs/position). The *maximum* amplitude of the positive peak
of each V1 VEP, and the minimum amplitude of the negative peak of each CA1 VEP were calculated. For each animal, these maximum and minimum values were transferred into Microsoft Excel and a specially derived array formula was used to compute the average amplitudes of the V1 and CA1 VEPs corresponding to each of the three visual stimuli (i.e., CS+, CS-, and Novel) in the front and side positions. First, a mixed model ANOVA was conducted to assess whether the post-acquisition Experimental Condition (i.e., Experiment I control, Experiment II control, Pilot) of the animals affected the visual evoked potentials recorded in the V1 and CA1. As post-acquisition Experimental Condition did not significantly affect visual evoked potentials, data from animals from different conditions were collapsed. For both the CA1 and V1, repeated-measures ANOVAs (within subjects factor: position – front and side, stimulus type) were used to compare whether the averaged amplitude values obtained from the front and side positions were similar in strength. This repeated-measures ANOVA also made it possible to compare the magnitude of VEP amplitudes elicited by the three visual cues (CS+, CS-, novel).
Chapter 4

Results

4.1 Visual Discrimination Task Acquisition for Experiment I and II

A total of 42 rats were trained in the visual discrimination task for Experiment I (n=18) and II (n=24). All rats successfully acquired the task, and an average of 10 days (Range: 5-17 days) was required to reach the performance criterion of 80% correct trials for three consecutive days. In order to evaluate whether the number of days taken to reach the performance criterion was related to the particular set of visual cues used for discrimination, a one-way analysis of variance (ANOVA) was conducted. The set of visual cues that the rats were randomly assigned to was the independent variable which consisted of 3 levels: Horizontal and Vertical, Diamond and Horizontal, Diamond and Vertical, and the number of days required to reach criterion was the dependent variable. From the results of the ANOVA, it was determined that animals given different sets of visual cues did not differ significantly in the number of days to reach performance criterion, $F(2, 39) = .614, p > .05$. This indicated that animals were able to discriminate between all visual cues to a similar degree of efficacy.

4.2 Experiment I

4.2.1 Experiment I Acquisition

As expected, during visual discrimination training, all rats (n=18) showed a decrease in the number of errors as training progressed. As shown in Fig. 5A, throughout the first 10 days of training, correct responses increased from about 50% (i.e., chance level) to about 80% (the training criterion). A repeated-measures ANOVA using Training
Training day as a within-subjects factor showed a significant effect of Training day, which was indicative of learning during the training phase of the experiment, $F(9, 9) = 17.61$, $p < .001$ (Figure 5A). Although rats were not separated into their respective experimental conditions during visual discrimination training, a mixed-model ANOVA was conducted to detect any significant pre-existing differences in acquisition performance between the saline and SC injected (n=12) and No Injection control (n=6) groups prior to pharmacological procedures (Figure 5B). The mixed-model ANOVA consisted of Training day as the within-subjects factor, and Post-acquisition pharmacological condition (saline and SC or No Injection) as the between-subjects factor. Results showed no significant effect of condition during the first 10 days of visual discrimination training, which suggested that both groups of rats performed similarly throughout acquisition, $F(1, 16) = .20, p > .05$. As expected, there was a significant within-subjects main effect of Training day which confirmed that rats from both conditions learned the discrimination task as training progressed, but no significant interaction of Post-acquisition pharmacological condition and Training day was found; main effect of Training day, $F(9, 18) = 13.82$, $p = .001$, interaction of Training day and condition, $F(9, 8) = .99, p > .05$. 
The mean acquisition performance presented in percent (%) correct across the first 10 days of visual discrimination training, error bars are displayed as standard error of the mean (±SEM). (A) The mean acquisition performance of all rats are shown (n=18). * According to follow-up pairwise comparisons, the acquisition performance on Day 1 and 2 differ significantly from each of the marked days (5-10), p’s < .05, which is indicative of learning. (B) The mean acquisition performance of all animals separated into their respective post-acquisition pharmacological conditions – No Injection Controls (n=6) and Saline and Scopolamine Injected (Ascending and Descending dose conditions combined; n=12).
4.2.2 Experiment I Post-acquisition

*Rats perform significantly worse when only one of the two visual cues is available*

After successful task acquisition, animals randomly assigned to the No Injection control condition (n=6) commenced 5 days of post-acquisition testing. On each day, 10 trials were administered, 8 of which were Regular Training trials (both visual cues present) and 2 were Probe trials (only one of the two visual cues present in the maze – CS+ for one probe trial and CS- for the other). As shown in Fig. 6, rats performed consistently better on Regular Training trials in comparison to Probe trials.

A repeated-measures ANOVA with the Trial Type (Probe or Regular Training trial) and Post-acquisition day (5 days) as within-subjects factors revealed a significant main effect of Trial Type, such that rats performed significantly better on Regular Training trials in comparison to Probe trials, $F(1, 5) = 12.08, p = .02$ (Figure 6). In contrast, neither the main effect of Post-Training day, nor the Post-Training day by Trial Type interaction was significant ($p$'s > 0.05). The significant main effect of Trial Type was followed up by computing pairwise t-tests, which showed that rats performed significantly better on Regular Training trials than on Probe trials on post-acquisition day 5, and marginally better on day 1 and 4 (see Appendix B - Table 1).
Figure 6. Experiment I Post-acquisition Performance under an Injection-free Condition

The mean performance on Regular Training Trials (2 visual cues available) and Probe Trials (either CS+ or CS- available) presented in % correct for Injection free rats (n=6) across 5 days of post-acquisition testing, error bars are displayed as standard error of the mean (±SEM). * indicates a marginally significant difference ($p < .09$), whereas ** indicates a significant difference ($p < .01$).

In the absence of Drugs or Injections, rats do not rely more heavily on either the positive or negative cue during Probe trials

Furthermore, I was interested in investigating whether animals relied more on the positive (CS+) or negative (CS-) visual cue during Probe trials in the absence of drugs or injections. An animal was thought to rely on a particular cue type if an error was committed on the Probe trial where that cue type was removed (i.e., if the animal made an error on a Probe trial with the CS- removed, it was inferred that the animal relied on the presence of the negative cue). In order to determine this, Probe trial performance of
all animals in the absence of each cue (Positive and Negative) was averaged and then collapsed across the 5 post-acquisition days. A Chi-square test examined if the reliance on each cue was significantly different than what could be expected based on chance. Results of the test were not significant, which indicated that in the absence of any drugs and injections, animals relied on the Positive and Negative cue equally, Pearson $\chi^2(4, N = 20) = 5.50, p >.05$ (Figure 7).

![Figure 7. Experiment I Probe trial Performance in No Injection Controls](image)

**Figure 7.** Experiment I Probe trial Performance in No Injection Controls

The mean performance on Probe trials with either the CS+ or CS– removed across 5 post-acquisition days (n=6), error bars are displayed as standard error of the mean (±SEM).

*The order in which rats receive saline and subsequent SC doses does not affect performance*

A further group of rats (n=12) received either ascending (n=6; saline, 0.125, 0.25, 0.5, 1.0 mg/kg SC) or descending (n=6; 1.0 mg/kg SC, 0.5, 0.25, 0.125, saline) doses of SC during the 5 days of post-acquisition testing. Initially, to assess whether the ascending
vs. descending drug regiments given to these two sub-groups of animals affected their performance differentially, a mixed-model ANOVA (between-subjects factor: Dose Order – Ascending or Descending, within-subjects factors: Drug Dose, Trial Type) was conducted. Results showed that the order in which animals received their injections of saline and SC did not significantly affect their performance, $F(1, 10) = 1.66, p > .05$. Thus, due to the fact that visual discrimination performance did not vary significantly as a result of dose order, the data from both sub-groups were collapsed for all further analyses.

**Regardless of saline and SC injections, rats perform better on Regular vs. Probe Trials**

Next, it was tested whether SC exerted significant effects on performance on Regular and Probe trials by conducting a repeated-measures ANOVA (within-subjects factors: Drug Dose, Trial Type). This ANOVA allowed for a comparison of visual discrimination performance when animals received saline (within-subject injection control) or different doses of SC, and the ability to detect any performance differences on the two different trial types. Results revealed a significant main effect of Trial Type, such that overall, animals performed significantly better on Regular Training trials in comparison to Probe trials, $F(1, 11) = 38.80, p < .001$ (*Figure 8*). Furthermore, the main effect of SC dose approached statistical significance, $F(4, 8) = 3.36, p = .068$, while the Trial Type by Drug Dose interaction was not significant, $F(4, 8) = .83, p > .05$.

Examining the main effect of Trial Type further, follow-up pairwise t-tests showed that rats performed significantly worse on Probe trials than Regular trials after receiving an i.p. injection of all doses of SC (0.125 – 1.0 mg/kg), but interestingly, Probe trial
performance was comparable to Regular trials after administrations of saline (see Appendix B – Table 2).

Figure 8. Experiment I Post-acquisition Performance after saline and varying doses of SC

The mean performance on Regular Training trials (2 visual cues available) and Probe trials (either CS+ or CS- available) presented in % correct of animals after receiving saline and varying doses of SC (n=12) across 5 days of post-acquisition testing, error bars are displayed as standard error of the mean (±SEM). * p = .01, ** p = .001.

It was not surprising that the main effect of dose was marginally significant as I noticed a slight, dose-dependent decline in Probe trial performance with increasing SC doses (Figure 8). In contrast, Regular trial performance remained unaffected, as animals continued to perform consistently above the criterion level (80%) at the highest SC doses. To examine these trends further, exploratory follow-up pairwise t-tests comparing the performance within each Trial Type were conducted separately. Results indicated that on Probe trials, performance after an administration of 1.0 mg/kg SC (see Figure 8) was
significantly worse than after an injection of saline, $t(11) = 3.07, p = .01$. In line with the
trend that could be seen in Fig. 8, the visual discrimination performance of rats on
Regular trials after receiving any dose of SC did not differ significantly from that of
saline.

**Regardless of SC dose, rats do not rely more heavily on either the positive or negative
cue during Probe trials**

Finally, I was interested in investigating whether the reliance on either the
positive or negative visual cue during Probe trials was influenced by different doses of
SC (*Figure 9*). To examine the relationship between cue reliance and SC dose, I
performed a Chi-square test that was composed of two variables: Drug Dose (saline,
0.125, 0.25, 0.5, 1.0 mg/kg of SC) and Cue Type Removed (Positive and Negative). This
analysis compared the *observed* number of incorrect responses associated with the
removal of each cue type (CS+ or CS-) to the *expected* number of incorrect responses at
each SC dose level (0 – 1.0 mg/kg). Results of the test were not significant, which
indicated that the type of cue an animal relied on was not significantly influenced by the
drug dosage it had received, Pearson $\chi^2(4, N = 43) = 2.86, p > .05$. 

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Figure 9. Experiment I Probe trial Performance after saline and various SC doses

The mean performance on Probe trials (only 1 visual cue available) when the CS+ is removed and when the CS – is removed after administrations of saline and varying doses of SC (n=12) across 5 days of post-acquisition, error bars are displayed as standard error of the mean (±SEM).

4.3 Experiment II

4.3.1 Experiment II Acquisition

As in Experiment I, a repeated-measures ANOVA with Training day as a within-subjects factor was conducted in order to examine performance changes during acquisition training. Results showed a significant main effect of Training day, such that visual discrimination performance in rats steadily improved across the initial 10 days of training, $F(9, 15) = 22.89, p < .001$ (Figure 10A). Again, although rats were not separated into their respective post-acquisition experimental conditions during acquisition training, a mixed-model ANOVA was conducted to detect any significant pre-existing differences
in acquisition performance between the Scopolamine (n=12) and No Injection control (n=12) groups prior to pharmacological procedures (Figure 10B). The mixed-model ANOVA (within subjects factor: Training day, between-subjects factor: Post-acquisition condition – No Injection or Scopolamine) revealed that performance during acquisition of the visual discrimination task did not differ significantly between the two post-acquisition conditions, $F(1, 22) = 3.85, p < .05$. As expected, a significant within-subjects main effect of Training day established that both groups of rats learned the discrimination task as training progressed, but there was no significant interaction of post-acquisition condition and Training day; main effect of Training day, $F(9, 14) = 24.81, p < .001$, effect of Condition by Training day interaction, $F(9, 14) = 1.74, p > .05$. 
The mean acquisition performance presented in percent (%) correct across the first 10 days of visual discrimination training, error bars are displayed as standard error of the mean (±SEM). (A) The mean acquisition performance of all rats are shown (n=24). * According to follow-up pairwise comparisons, the acquisition performance on Day 1 and 2 differ significantly from each of the marked days (5-10), *p’s < .05 in a way that is indicative of learning. (B) The mean acquisition performance of all animals separated into their respective post-acquisition pharmacological conditions – No Injection Controls (n=12) and Scopolamine (n=12).
4.3.2 Experiment II Post-acquisition

After reaching task criterion, rats were randomly assigned to one of two conditions for post-acquisition testing, No Injection Controls or daily administrations of 1.0 mg/kg SC. On each of the 5 days of post-acquisition testing, animals were assessed on their performance on 8 Regular trials and 2 Probe trials. As shown in Fig. 10A, regardless of post-acquisition drug condition, performance on regular trials was consistently at or above the acquisition criterion (80% correct) throughout the 5 days of testing. In contrast, for rats receiving daily injections of 1.0 mg/kg SC, performance overall on Probe Trials was impaired relative to No Injection controls.

In order to compare visual discrimination performance on Probe and Regular Trials between the two pharmacological conditions (No injection and Scopolamine – 1.0 mg/kg) across 5 days of post-acquisition testing, a mixed-model ANOVA (within-subjects factors: Trial Type – Regular and Probe trial, Post-acquisition day – 5 days, between-subjects factor: Post-acquisition condition – No Injection and Scopolamine) was conducted. This analysis showed a significant between-subject effect of Post-acquisition condition, such that based on overall visual discrimination performance (not accounting for the particular Trial Types involved), No injection controls significantly outperformed SC injected animals, $F(1, 22) = 17.61, p < .001$. Furthermore, results indicated a significant main effect of Trial Type (Regular trial performance better than Probe trial), and interaction of Trial Type and Post-acquisition condition; effect of Trial Type, $F(1, 22) = 74.12, p < .001$; Trial Type by condition interaction effect, $F(1, 22) = 18.37$, $p < .001$. In other words performance on the two trial types differ significantly, and this difference is influenced by the post-acquisition pharmacological condition that the
animals are assigned to. No significant main effect of Post-acquisition day, or interactions between: day and condition, trial type and day, trial by day by condition were found, $p$’s > .05.

*Rats injected with SC perform worse when only one cue is available while performance on trials with both cues remains unaffected*

Although a significant main effect Post-acquisition condition was found, the initial mixed-model ANOVA did not differentiate between whether the two conditions performed statistically different on both Regular and Probe trials or just one trial type. To address this, one additional mixed-model ANOVA was computed to compare performance between the No Injection controls and SC injected animals on Regular trials, while another mixed-model ANOVA examined performance between conditions on Probe trials. Interestingly, these separate analyses indicated that although Regular trial performance between conditions did not differ significantly (i.e., despite SC injections, performance on Regular trials did not differ from controls; Figure 10A), SC injected rats performed significantly worse than No Injection controls on Probe trials (Figure 10B); Regular trial performance, $F(1, 22) = 2.73, p > .05$, Probe trial performance, $F(1, 22) = 21.75, p < .001$. To investigate the significant difference in Probe trial performance found between the two conditions further, follow-up mixed-model t-tests were conducted using various output terms provided by the ANOVA. These tests revealed that No Injection control animals significantly outperformed SC animals on Probe trials on all post-acquisition days ($p$’s < .05; Figure 9A; for specific t-test values see Appendix C-Table 1).
Figure 11. Experiment II Post-acquisition Regular and Probe trial Performance

The mean acquisition performance presented in percent (%) correct across 5 days of post-acquisition testing, error bars are displayed as standard error of the mean (±SEM). (A) The mean Regular Trial performance of No Injection controls (n=12) vs. Scopolamine Injected rats (n=12). (B) A comparison of the mean Probe trial performance of No Injection controls to Scopolamine Injected rats. * $p < .05$, ** $p < .01$.

4.4 Visual Evoked Responses in Hippocampus and Visual Cortex

The day immediately following the last day of post-acquisition testing (Day 5), No Injection control animals from Experiment I (n=6) and II (n=12), and a subset of animals from the Pilot Experiment (n=14) were prepared for surgery and electrodes were
lowered into the V1 and CA1 to record visual evoked potentials elicited by visual stimuli (Figure 12). Under anaesthesia, 100 separate recordings were taken for each stimulus (CS+, CS-, and novel) in both the Front (monitor directly in front of the rat) and Side (monitor to the left of the rat, in line with the left eye) positions. Although a total of 32 animals underwent electrophysiological procedures, 6 were discarded due to incorrect placements, and all analyses reported here were conducted on the recordings of 26 rats.

![Diagram of V1 and CA1 responses](image)

**Figure 12. Typical Visual Evoked Response in V1 and CA1**

Typical VEPs elicited by familiar visual stimuli encountered during training (average of 200 waveforms evoked in response to stimulus presentation) measured simultaneously in V1 (A; red) and CA1 (B; blue) across 250 milliseconds. The arrows indicate the stimulus onset. The maximum amplitude of the positive peak and the minimum amplitude of the negative peak were taken for each waveform and used to compile average amplitudes for the V1 and CA1, respectively, for each stimulus.
Differences in Post-acquisition procedures do not affect Visual Evoked Potentials

Initially, to assess whether the post-acquisition Experimental Condition (Experiment I controls, Experiment II controls, and Pilot Experiment animals) affected visual evoked potential amplitudes measured in the primary visual cortex (V1) and area CA1 of the hippocampus, a mixed-model ANOVA (between-subjects factor: Experimental Condition, within-subjects factors: Monitor Position, and Stimulus Type) was conducted. Results showed that in both the V1 and CA1, the visual evoked potential amplitudes measured in animals from different Experimental Conditions did not differ significantly; $F(2, 23) = 0.84, p > .05, F(2, 23) = 3.06, p > .05$, respectively. Thus, the data from all animals were collapsed for all further analyses.

Visual evoked potentials are stronger when stimuli are presented to the Side vs. Front

When comparing the evoked potential amplitudes measured in the primary visual cortex (V1; Figure 13) and hippocampus (CA1; Figure 14), the evoked responses appeared slightly larger when the visual stimulus was flashed to the left side of the animal (Side) versus to the front of the head (Front). To statistically compare the strength of visual evoked responses taken from the Front and Side, two repeated-measures ANOVAs (one for V1 another for CA1) with Stimulus Type (CS+, CS-, Novel) and Monitor Position (Front and Side) as within-subject factors were computed. In V1 the visual evoked responses recorded from the Front and Side were not found to be significantly different; V1, $F(1, 25) = 1.50, p > .05$. In the CA1, however, the VEPs induced by stimuli shown from the Side position were significantly greater than the VEPs elicited by stimuli presented from the Front, $F(1, 25) = 5.86, p < .05$. 

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Figure 13. Evoked Responses in the V1 to Visual Cues Presented from the Front and Side

The mean visual evoked potential amplitude in mV recorded in the Primary Visual Cortex (V1) in response to visual stimuli presented to the left side of the animal, in front of the left eye (Side), and directly in front of the animal (Front), error bars are displayed as standard error of the mean (±SEM).

Figure 14. Evoked Responses in the CA1 to Visual Cues Presented from the Front and Side

The mean visual evoked potential amplitude in mV recorded in the Hippocampus (CA1) in response to visual stimuli presented to the left side of the animal, in front of the left eye (Side), and directly in front of the animal (Front), error bars are displayed as standard error of the mean (±SEM).
VEPs elicited by the Positive, Negative, and Novel Stimuli were not significantly different in V1 and CA1

Next, I was interested in determining whether the VEPs elicited by the different stimulus types (CS+, CS-, Novel) in V1 and CA1 were different. The repeated-measures ANOVA computed for the V1 revealed that there was no significant main effect of Stimulus Type, such that the VEPs for all three stimulus types were not significantly different, $F(2, 50) = 1.30, p > .05$. Due to the significant main effect of position in the CA1 VEPs, a repeated-measures ANOVA (within-subjects factor: Stimulus Type) for each position (Front and Side) was conducted to compare the VEPs pertaining to each stimulus type. Regardless of whether the stimulus were presented from the Front or Side positions, the VEP measured in the CA1 in response to each Stimulus Type were not significantly different; Front: $F(2, 50) = .32, p > .05$, Side: $F(2, 50) = .83, p > .05$. Not surprisingly, in all analyses there was no significant interaction effect of Monitor Position and Stimulus Type ($p$’s > .05).
Chapter 5

Discussion

The first objective of this thesis was aimed towards gaining a better understanding of the role of acetylcholine in mediating performance in a learning and memory task. Through a visual discrimination paradigm, I attempted to discern whether previously documented performance impairments (across a variety of tasks) associated with ACh muscarinic receptor blockade, were predominantly due to the importance of muscarinic receptor integrity in attention, or memory encoding and consolidation in learning. To address the second objective, electrophysiology recordings were used to further examine the experience-dependent changes in V1 and CA1 resulting from extensive visual discrimination training. In particular, I was interested to see whether the stronger visually-evoked responses in V1 to familiar visual stimuli reported by Hager and Dringenberg (2010) could also be measured in area CA1 of the hippocampus.

5.1 Experiment I

5.1.1 Discussion of Results

In Experiment I, the platform was removed during Probe trials (only 1 visual cue rather than 2) in an effort to prevent reinforcement and subsequent acquisition of a new strategy to solve single cue-guided discriminations. By limiting opportunities for further learning (no reinforcement for correct discriminations), I argue that performance on these trials provided a way to measure attentional processes involved in this type of trial. For this experiment, visual attention was defined as the ability of an animal to select task-
relevant information from the particular visual stimuli presented (during a Probe trial) to localize the hidden escape platform (even though it was absent in this case).

Results demonstrated that, in the absence of one of the visual cues, performance was impaired relative to Regular trials (both cues present). Also, it appeared that performance could be further impaired by decreasing ACh muscarinic receptor availability, even though the dose-dependent decrease in Probe trial performance did not reach statistical significance. It is crucial to note that the negative impact of higher SC doses on Probe trial performance could not be attributed to the peripheral side effects of the drug (e.g., pupil dilation, increased locomotor activity), as Regular trial performance was maintained above criterion regardless of SC dose. With regards to the importance of cue-type (CS+ and CS-), both groups of animals did not differ in their reliance on either cue, and seemed to depend on the presence of both the positive and negative cue equally.

The finding that rats performed worse on Probe trials in comparison to Regular trials is consistent with my hypothesis. As this effect was present in animals that had never been exposed to drugs or injections, it is fair to assume that the negative impact on performance may have resulted from an increase in task difficulty, when visual information was reduced. Generally, as a task becomes more challenging (e.g., increasing distracters, more subtle differences between stimuli) the demands on attention become greater, and thus, response latency and the number of errors often increase (e.g., Eriksen & Hoffman, 1972; Grady et al., 1996; Robbins, 2002). One may argue that when only one visual cue is presented, performance should improve rather than worsen because the rat must only attend to information provided by one stimulus, rather than two. However, in this task, I propose that the information provided by the two types of visual cues are
consistent and complimentary, and therefore, the presence of a second cue reaffirms the information gathered from a single cue presentation. The hypothesis that the presence of two visual cues provides consistent, complimentary information is further strengthened by the fact that Probe trial performance was similar, regardless of whether the CS+ or CS- was removed. Although I had hypothesized that performance would be worse in Probe trials with the CS+ absent, the results indicated that rats did not disregard the information provided by the CS-. Instead, rats seemed to rely on both cues equally and their superior performance on Regular trials reflected their preference for having an additional source of information (2 cues instead of 1).

In a quest to find better ways of measuring and understanding attention, a surge of research in the past decade has been devoted to examining the neural mechanisms that underlie this process (Knudsen, 2007). Brain-imaging and neurophysiological data across many studies suggest that attention is not governed by a single brain area, nor does it involve the entire brain (see review by Posner & Dehaene, 1994). According to Posner and Dehaene (1994), the cortical areas that experience a relative amplification of blood flow and neuronal firing as a result of increased attention are task-dependent. Given the fact that I employed a visual discrimination task in this study, the areas of the brain associated with visual attention will be the focus of this discussion.

From a detailed review of the findings from various brain imaging studies, Kanwisher and Wojciulik (2000) reported that, not surprisingly, visual attention affects the first stage of cortical information processing, the primary visual cortex. Furthermore, according to these researchers, large regions within the fronto-parietal network support a variety of visual attention tasks. In fact, the frontal and parietal lobes have been proposed
as origins of the amplification signals associated with attention (Posner & Dehaene, 1994), and as such, have been implicated in the direction of visual attention (Kanwisher & Wojciulik, 2000). Interestingly the (pre)frontal, parietal, and visual regions of the brain – which have been deemed most important for intact visual attentional processing in both humans and other animals, are areas in which ACh plays a vital role in the top-down control of attention orientation and stimulus discrimination (Klinkenberg, Sambeth, & Blokland, 2010). Furthermore, a collection of structures in the basal forebrain (e.g., nucleus basalis, medial septal nuclei) are considered a major cholinergic output of the CNS and are important for the production and distribution of ACh throughout the brain (see review by Cuello & Sonfroniew, 1984).

Using microdialysis techniques, researchers found increases in medial prefrontal cortical (mPFC) ACh levels during performance of an attention task (5-choice serial reaction time task: 5-CSRTT; Passetti, Dalley, O’Connell, Everitt, & Robbins, 2000). Moreover, Dalley and colleagues (2001) showed an elevated ACh release in the prelimbic PFC region in rats performing a visual attention task relative to animals that received a control version (stimulus detection response was non-contingent with reward). In addition to a similar increase in fronto-parietal ACh measured during performance in attention paradigms, Himmelheber, Sarter, and Bruno (2000) noticed that the amount of ACh efflux was related to the level of attentional demand. Himmelheber, Sarter, and Bruno (2000) found that augmentation of the attentional demand produced by a distracter stimulus, elicited further increases in ACh release. Further support for the notion that ACh release modulates (attentional-related) sensory processing is provided by the observation that nucleus basalis stimulation (known to increase ACh in the neocortex)
resulted in a decorrelation of neuronal activity in V1, indicative of more precise, local sensory processing (Goard and Dan, 2009).

With these past findings in mind, my results are not only consistent with my earlier hypotheses, but they seem to fit well with existing literature. Although I did not find a significant main effect of SC dose on Probe trial performance (e.g., performance after 0.125 mg/kg SC did not differ significantly from performance after 1.0 mg/kg), a clear decrease in performance was observed with higher SC administrations. Notably, rats exhibited a significant impairment in Probe trial performance after 1.0 mg/kg of SC in comparison to performance after an administration of saline (within-subject injection control). From these results, I believe that the decrease in muscarinic ACh receptor availability was largely responsible for impairing normal cholinergic functioning, which was important for sustaining the level of visual attention needed for rats to perform during Probe trials. Furthermore, if one takes into account the previous finding that the amount of ACh efflux was related to the difficulty level of the task, a decrease in muscarinic receptors would prevent the action of additional ACh released in response to the more challenging Probe trials (as proposed earlier). As such, without the increased cholinergic activity brought on by ACh, it is plausible that the poorer performance on Probe trials was a result of impaired visual attention.

Similar impairing effects of SC on attention have been documented in a number of other studies including Bushnell, Oshiro, and Padnos (1997), and Robbins et al. (1998). After subcutaneous injections of SC (0.03, 0.056, and 0.1 mg/kg), Bushnell and colleagues (1997) noted a significant decrease in correct responses and an increase in: reaction time, false alarms (respond during non-signal trial), and omissions, relative to
baseline performance (before SC administrations) in a visual-signal detection task.

Moreover, disruptions in performance (attention) were induced with direct, local administration of SC in an experiment conducted by Robbins et al. (1998). Local infusions of 10 µg/site of SC into the mPFC (unilaterally) and anterodorsal lateral frontal cortex (bilaterally) of rats produced a significant reduction in accuracy and an increased response latency in the 5-CSRTT (Robbins et al., 1998). Surprisingly, in the present study, systemic SC administration (0.125–0.5 mg/kg) did not impair performance, even though other work has found impairing effects at doses lower than those used here.

With noticeable impacts on attention seen at such low doses of SC (.03 – 0.1 mg/kg), it may seem odd that the relatively higher doses of SC used in this experiment did not produce significantly poorer Probe trial performance when compared to saline. A possible explanation for this finding could be based on the differences in attention and sensory processing involved for each distinctive paradigm. Perhaps my particular visual discrimination task required a different form of attentional processing than the signal-detection and 5-CSRT tasks, with visual discrimination not as readily impaired by lower doses of SC. The different routes of SC administration employed by experiment could have also accounted for this disparity in dose effectiveness. Robbins et al. (1998) infused SC directly in certain areas of the brain thought to be involved in attention, which would have resulted in an immediate, localised action of the drug. Bushnell et al., (1997) on the other hand, delivered the SC doses subcutaneously, but did not specify which area of skin was selected for the injection. In my experiment, I chose an intraperitoneal method of administration, which would have resulted in a more widespread frame of drug activity, and/or differences in drug concentration in the central nervous system. Due to the high
level of SC dispersion from peripheral injection sites to the brain, drug potency could have been greatly reduced when the anticholinergic activity finally reached susceptible targets. Therefore, despite administrating relatively higher doses in comparison to the other two experiments, the degree of ACh muscarinic receptor blockade in important brain areas for attention was likely less effective, which could account for the lack of significant impairments on Probe trial performance at 0.125 - 0.5 mg/kg of SC.

5.1.2 Limitations and Confounds

Together, the significantly poorer performance on Probe trials relative to Regular trials across post-acquisition days, and further impairment on Probe trial but not Regular trial performance after 1.0 mg/kg of SC, point towards a greater attentional demand when information is reduced, and the importance of ACh muscarinic receptor functioning in sustaining attention, respectively. Although these interpretations of my experimental results are logical and supported by relevant literature, the most obvious and crucial limitation of this experiment is an inability to confirm the cognitive function that was assessed through Probe trial performance. In other words, there was no way to ascertain that Probe trial performance was a measure of visual attention, and as such, the conclusions drawn about the importance of ACh in attention could have been misleading.

Perhaps performance on Probe trials was not an examination of how well the animal could attend to given stimuli, but rather, a test of how readily they could recall the platform association made with each cue type (therefore a memory task). It is also possible that the observed decrease in Probe trial performance was simply an effect of how rats responded to novelty. As the animal’s first exposure to this type of single-cue
trial coincided with the start of post-acquisition testing, rats may have been more preoccupied with exploring its surroundings (after noticing the change in apparatus appearance) than locating the hidden platform. However, consistently poor Probe trial performance in controls animals across the 5 post-acquisition days is strong evidence against the likelihood that performance impairments were due to novelty alone.

Another confounding variable in this experiment that cannot be entirely ruled out is the anxiogenic side effect of SC. In studies measuring the time animals spent in dark and bright compartments of an open field, SC doses ranging from 0.05 – 2 mg/kg were found to decrease the number of transitions to the bright side (indicative of increased anxiety), and increase anxiety related behaviours (Hughes, Desmond, & Fisher, 2004; Smythe, Murphy, Bhatnagar, Timothy, & Costall, 1996). Although the inclusion of Regular trials during post-acquisition testing was intended to control for most side effects (e.g., increased locomotor activity and pupil dilation), the impairing influence of anxiety on performance could have been masked during Regular trials, due to the hypothesized ease of the task when both cues were present. According to O’Brien and Crandall (2003), women scored better on an easy math test, but worse on a difficult one, when they were more anxious. Consequently, the poorer performance in rats that received 1.0 mg/kg of SC could have resulted from a combined effect of task difficulty and increased anxiety, instead of disruptions in visual attention.

Finally, as I had administered SC in an intraperitoneal fashion, the widespread anti-muscarinic receptor effect on different brain areas was far from localized. Without the confidence of a targeted frame of anti-cholinergic action (e.g., only in V1, forebrain area, etc), it is impossible to report conclusively that Probe trial performance was a
reflection of muscarinic receptor blockade in areas of the brain responsible for sustaining visual attention. The impairment in Probe trial performance could have been a result of SC effects on brain areas completely unrelated to attention. Therefore, in future experiments, SC could be infused directly to certain brain areas of interest through implanted cannulas, a procedure that would aid in avoiding this ambiguity.

5.1.3 Future Directions

First, to address the major limitation involving the inability to ascertain whether Probe trial performance was an accurate measure of visual attention, I have proposed a few alterations and extensions to the current methods. As discussed earlier, in addition to performance, increased response latency has often been used as a measure of attention. Therefore, for future experiments, the time taken to make a decision (i.e., time for more than half of the body to enter a goal arm) for each Probe and Regular trial during post-acquisition testing should be recorded along with performance, as an added measure of visual attention. Response latency could also provide another way to measure whether a difference in reliance on the two cue types exists (e.g., if an animal takes longer to respond when a certain cue type is removed, it can be inferred that it relies more strongly on its presence). Furthermore, in an attempt to make the task more attentionally demanding, rats could be trained using visual cues that are quite similar, making the discrimination more challenging. Therefore, during Probe trials, when only one cue is presented, the animal will need to employ a greater attentional effort to decide which goal arm to swim towards. Another option would be to have the visual cues presented on flat screen monitors at the end of each goal arm to allow the experimenter to make the task
progressively more difficult. An example of this would be to lower the contrast of presented visual cues, or have the cues appear more faintly, to encourage the animal to attend more closely to the stimuli presented.

Another task modification could involve adding a biomotion sensor to the Y-maze apparatus would cause one of the visual cue(s) to disappear once the animal had swum past a preset distance threshold. Under this circumstance, animals would have to attend carefully to visual stimuli as they approached the goal arms because the cues would not be available to guide their decision once they crossed a certain point. Finally, rats participating in post-acquisition visual discrimination testing could also be trained on another task designed to access attention, such as the 5-CSRTT. The performance on the two tasks could then be compared to see if a correlation existed, and these results could help us infer the validity of Probe trial performance as a measure of attention. Alternatively, the experimenter could combine the performance on both tasks to create an “averaged score” for each animal, which would likely be a better measure of attention than performance on either task alone.

In order to explore whether ACh muscarinic receptor integrity specifically, or the action of ACh in general (importance of both nicotinic and muscarinic receptors) is important for visual attention, future studies could employ a nicotinic receptor blocker in this task (e.g., mecamylamine). To identify brain areas mediating nicotine-induced attentional enhancement, Hahn, Shoaib, and Stolerman (2003) injected nicotine (0, 1, 2, 4, and 8 µg/site, bilaterally) into the dorsal hippocampus and prelimbic area of the PFC in rats that were tested in the 5-CSRTT. Nicotine injected into the dorsal hippocampus had no effect on any measure of performance other than a slight decrease in response latency
in some animals at lower doses. In contrast, local injections of nicotine into the prefrontal cortex resulted in a dose-related increase in accuracy, but no change on measures reflecting sensorimotor activation (i.e., rate and speed of responding). From these findings, the researchers concluded that the prefrontal cortex, but not the dorsal hippocampus, was important for the attention-enhancing effects of nicotine. A recent study that examined the nicotinic antagonistic effects on functional attentional networks administered mecamylamine to healthy male subjects performing the Attention Network Task (ANT) in an MRI scanner (Thienel et al., 2009). In comparison to a placebo, mecamylamine was found to slow overall response time and down-regulate brain activation associated with orienting, and to some extent, brain activation associated with executive control. Despite evidence from these studies implicating nicotinic involvement in attentional processing, Herrerro and colleagues (2008) reported findings that appeared to suggest otherwise. Herrerro et al. (2008) found that attending to the particular receptive field of a V1 neuron caused an increase in firing rate, which was further enhanced by ACh (low doses), and modulated by SC, but not by mecamylamine. Therefore, in light of this seemingly contradictory finding of the importance of nicotinic receptor mechanisms in cholinergic attentional modulation, it would be highly worthwhile to examine the effects of mecamylamine in my visual discrimination paradigm.

5.1.4 Conclusions

The results of Experiment I compliment the findings of previous work examining the importance of cholinergic functioning – in particular, muscarinic receptor integrity, in
attention. In the absence of drugs or injections, rats performed notably worse when only one visual cue was available (Probe trials), which indicated their preference of having two sources of information, and raised the possibility that single cue-guided trials were more challenging and attentionally demanding. In line with this notion, Probe trial performance was found to be further impaired after 1.0 mg/kg of SC, a muscarinic receptor antagonist. As such, I proposed that the disruption in visual attention processing brought on by anti-muscarinic receptor activity, was responsible for the added impact on a rat’s ability to perform during Probe trials. The findings from this experiment provide evidence for the importance of centrally-acting ACh muscarinic receptors in modulating visual attention functioning.

5.2 Experiment II

5.2.1 Discussion of Results

In order to provide animals with reinforcement for correct decisions made during trials with only one visual cue, the platform was present during the Probe trials in Experiment II. I reasoned that by allowing an association to form between correct performance and platform localization, I was giving rats an opportunity to acquire a new strategy used to solve single cue-guided discriminations. By comparing the Probe trial performance of No injection controls and animals given 1.0 mg/kg SC, I hoped to investigate the role of ACh muscarinic receptors in the memory encoding and consolidation processes involved in learning a new strategy.

As hypothesized, results demonstrated that rats receiving daily injections of 1.0 mg/kg SC performed significantly worse during Probe trials on each post-acquisition
day, relative to No injection controls. As shown in Fig. 11 (see pg 49), control animals started out at about 70% correct, but rapidly reached the criterion level (80%) of performance by post-acquisition day 2, and maintained at that level for the next 3 days. In contrast, SC animals achieved 45% correct on the first day, and did not improve any higher than 65% correct throughout the subsequent post-acquisition days. As in Experiment I, the differences in Probe trial performance could not be attributed to the peripheral side effects of SC because the Regular trial performance of both groups of animals was almost identical.

Based on these findings, it appears that control animals rapidly reached, and maintained a high level of performance on Probe trials because they were able to readily learn and acquire the new, single-cue strategy without interference. However, with the blockade of ACh muscarinic receptors in the brain by SC, it is likely that rats were unable to properly encode and consolidate the memories needed to learn the new strategy. Therefore, despite having the platform reinforcement during all Probe trials, rats continued to perform poorly because the normal functioning of ACh muscarinic receptors was essential for acquiring new information. On the other hand, performance on Regular trials was unaffected by administrations of SC, likely because acquisition and consolidation of the visual discrimination task involving 2 cues had already taken place, before disruptions in muscarinic receptor activity.

In the context of existing literature, my findings are consistent with studies that suggest muscarinic cholinergic receptor blockade by drugs (e.g., Scopolamine) impairs the encoding of new memories, but not the retrieval of previously learned information (Hunter & Murray, 1989; Atri et al., 2004). When SC was administrated before trials...
commenced, male rats tested in a simple olfactory habituation and discrimination paradigm failed to habituate to the first odour, and were unable to recognise and respond appropriately to the second (novel) odour (Hunter & Murray, 1989). However, when SC was introduced after the third trial, rats performed like saline and methyl-scopolamine treated animals; that is to say, they habituated to the familiar odour and investigated the novel odour, which implied that the rats had an intact memory for the previously presented odour. Hunter and Murray (1989) were also keen to point out that, because rats were still able to increase their responding to the new stimuli, it was unlikely that SC affected the animal’s ability to properly attend to, and discriminate between stimuli.

Similar findings were presented in a recent paper by Atri et al. (2004), which investigated the effect of central cholinergic receptors blockade on word-pair recall in healthy young humans. Compared to subjects that received placebos or no injections, individuals given SC (8 µg/kg) showed an overall impairment for new word paired-associate learning, but exhibited no problems recalling previously learned word associates.

There is a general consensus among many researchers that learning and memory may depend on the collective contribution of multiple neural systems that process different features of a given experience (Poldrack & Packard, 2003; White & McDonald, 2002). Despite the distinction often made regarding the role of the medial temporal lobe (e.g., hippocampus) in supporting “declarative” memory (e.g., Cohen & Squire, 1980) and the basal ganglia (e.g., dorsal striatum) in governing “habit/procedural” memory (e.g., Packard & Knowlton, 2002), most learning situations require an interactive participation of many neural systems (Gold, 2003). According to a review by Gold (2003), ACh, together with other neurotransmitters and modulators (e.g., biogenic
amines, steroids and peptides), may modulate learning and memory by altering the balance in contribution of these different systems. Gold also points out that various pharmacological effects on learning and memory are closely associated with ACh release in the hippocampus, striatum, and amygdala. Furthermore, assessment of ACh output in different brain areas during learning has been predictive of individual rates of learning in some tasks (Gold, 2003).

In light of the large body of research outlining the very close relationship that appears to exist between ACh and learning and memory, we may be tempted to draw the conclusion that ACh has a unique role in the regulation of these processes. However, as discussed in earlier sections, it is important to take into account the extensive literature identifying the essential neuromodulatory functions of ACh in attentional processing, before making such conclusions. In fact, some researchers have become so convinced of the role of ACh in attention, that they have begun intimating that attention impairments may underlie the deficits in learning and memory that often result from disruptions in cholinergic functioning (e.g., Blokland, 1995; Klinkenberg & Blokland, 2010). From their point of view, decreases in cognitive performance after ACh blockade may not reflect difficulties in memory encoding and consolidation per se, but rather, a consequence of being unable to properly attend to presented information. In support of this idea, researchers have brought forth breakthrough findings involving 192 IgG-saporin, a highly selective toxin for ACh neurons. Even though the immunotoxin resulted in a near-total loss of ChAT-positive (Choline acetyltransferase-positive) neurons in the medial septum (accompanied by decreases in markers for cholinergic function within the hippocampus), major impairments in learning and memory were surprisingly absent.
(Baxter & Murg, 2002). As such, this lack of impairment has raised serious questions about the importance of septohippocampal cholinergic neurons in mediating learning and memory processes (e.g., Baxter & Murg, 2002).

Although these findings appear to constitute compelling evidence against cholinergic modulation of learning and memory, results from experiments conducted by Chang and Gold (2004) have provided plausible alternate explanations. Chang and Gold reported that 192 IgG-saporin injected into the medial septum/vertical limb of the diagonal band (MS/VDB) produced a near-total loss of ChAT-positive neurons similar to Baxter and Murg (2002), but interestingly, this loss was accompanied by only a partial decrease (to 40% control levels) in ACh release in the hippocampus. Moreover, when saporin-lesioned and control rats were tested in a spontaneous alternation task, the resulting percent increase in hippocampal ACh was comparable between the two groups. Thus, the ACh release in saporin-lesioned animals indicated residual cholinergic function in the hippocampus (even after marked ChAT-positive neuron loss) and a level of responsiveness to memory tests similar to controls. Based on these results, Chang and Gold (2004) proposed that previous studies examining the consequences of ChAT-positive cell loss after 192 IgG-saporin induced lesions, may have overestimated the extent of disruption to septohippocampal ACh functions. Furthermore, they speculated that post-damage, neural reorganization could possibly engage compensatory mechanisms (such as those seen in acetylcholinesterase knockout mice – Li, Duysen, Volpicelli-Daley, Levey, & Lockridge, 2003) sufficient enough to help restore learning and memory processes in lesioned-animals. Therefore, when viewed in this context, the lack of learning and memory impairments in animals injected with 192 IgG-saporin is
less surprising and does not rule out cholinergic mechanisms involved in learning and memory.

Another valid problem with interpreting performance impairments as a measure of learning and memory deficits is the difficulty (sometimes impossibility) in separating the effects of memory from those of attention. During the acquisition phase of many tasks, mechanisms of attention and learning are at work (and may interact) simultaneously, and the distinctions between “cognitive taxonomy” become understandably blurred (Gold, 2003). Importantly, however, specific experimental strategies such as post-training designs have made it possible to identify the effects on memory apart from effects on attention, motivation, and arousal. From a review of the literature involving cholinergic treatments administered after training, we can conclude that cholinergic activation via muscarinic receptors is indeed a critical component in the modulation of memory consolidation (Power, Vazdarjanova, & McGaugh, 2003).

Bringing the focus back to my results in Experiment II, although I could not completely tease out the effect of SC on the learning process of a new strategy from attentional processes, I believe that the anti-muscarinic disruptions in memory encoding and consolidation contributed significantly to the performance impairment on Probe trials in SC animals. Despite my confidence in this interpretation, I have come to realise that it is impossible to identify a singular role of ACh, especially in broad cognitive processes such as attention, and learning and memory. Behavioural, pharmacological, and electrophysiological evidence have all shown that ACh release may reflect modulation of learning and memory processing, arousal of several neural systems during learning, or activation of attentional mechanisms (Gold, 2003). Moreover, it is highly unlikely that a
neurotransmitter with such widespread brain distribution like ACh has a unitary cognitive function. Therefore, in agreement with Gold (2003), I conclude that ACh may be involved in regulating neocortical arousal, selecting stimuli for further processing, allowing for behavioural flexibility, and modulating temporal processing in a manner important to learning and memory, as well as directly supporting memory consolidation processes.

5.2.2 Limitations and Future Directions

As is the case with a number of studies, the major limitation in Experiment II was an inability to segregate SC effects on attention from those on memory encoding and consolidation in learning. Despite providing platform reinforcement during Probe trials to add a learning component to a task initially designed to assess attention, my method of assessing SC effects on memory did not take into account the mechanisms of attention that could have also been affected. I could prevent this in a future study by changing the route, and more importantly, the timing of SC administration. By infusing SC directly into cannulas implanted in areas where ACh muscarinic receptors are thought to be important for memory consolidation such as the basolateral amygdala (see review by Power, Vazdarjanova, & McGaugh, 2003) and the hippocampus, I may be able to minimize the widespread blockade in other areas (that may affect performance, but are not specifically involved in memory consolidation). Furthermore, by administering SC immediately after post-acquisition testing instead of 30 minutes before, I will be able to rule out anti-cholinergic effects on attention, and examine the importance of muscarinic receptor integrity during consolidation of a new strategy.
Another potential confound that could also be applied to Experiment I, was the method in which Probe trial performance was assessed. Unlike the high number of Regular trials (total of 8), animals were only given 2 Probe trials a day, which greatly reduced the number of experimental measures that could be obtained from each animal. This method of assessing Probe trials also meant that the difference between getting one trial correct versus two resulted in a performance difference of 50%, whereas in Regular trials, making a correct discrimination on one trial only resulted in a performance change of 12.5%. In order to provide more opportunities to accurately assess Probe trial performance, each rat should be given a total of 4 Probe trials (2 for each cue type – CS+ and CS-) along with 8 Regular trials. This would allow me to gather more information about single cue-guided performance, while not overtaxing the animals physically through the incorporation of too many trials.

5.2.3 Conclusions

The results of Experiment II are in line with previous findings suggesting that muscarinic receptor blockade is responsible for impairing the encoding and consolidation of new memories, but not the retrieval of previously acquired information. Rats given daily administrations of 1.0 mg/kg of SC prior to testing exhibited significantly worse Probe trial performance relative to No Injection controls, across all 5 post-acquisition days. As both groups of animals performed almost identically on Regular trials, I concluded that the peripheral side effects of SC were not responsible for Probe trial impairment, and that muscarinic receptor integrity was not important for strategies that had already been learned and consolidated (visual discrimination using 2 cues).
5.3 Visual Evoked Responses in Hippocampus and Visual Cortex

5.3.1 Discussion of Results

The electrophysiology portion of this thesis demonstrated that the responses elicited by familiar (encountered during visual discrimination training: CS+ and CS-) and novel visual stimuli, measured in the primary visual cortex (V1) and area CA1 of the hippocampus, were not significantly different from one another. Furthermore, although the evoked responses appeared to be larger when the visual stimulus was presented directly in front of the left eye of the animal (Side) versus the front of the head (Front), this difference only reached statistical significance in the hippocampus CA1 area.

Contrary to my hypothesis, responses measured in the V1 were similar regardless if they were elicited by familiar or novel visual stimuli. Notably, these results are inconsistent with the greater neuronal response to familiar stimuli at early stages of visual processing (V1) reported by Hager and Dringenberg (2010), and appear to contradict earlier fMRI research that revealed increased overall responses to trained stimuli in the human V1 (Furmanski, Schluppeck, & Engel, 2004). Although surprising at first, there are a few factors that could have accounted for this difference. To begin, it is important to point out that even though Hager and Dringenberg found enhanced responses to stimuli encountered during training relative to a novel visual cue, this difference was extremely small (~ .05mV). Moreover, the sample size (n=24) used in their experiment was large by usual electrophysiology research standards, which may be an indication that many subjects were needed to increase the power sufficiently to pull out this effect.

It is also worthwhile to mention that although I used the same visual discrimination training procedure as Hager and Dringenberg, my pre-surgical methods
differed slightly with the addition of a post-acquisition testing phase. It is possible that a combination of continued exposure of animals to visual discrimination training (Regular trials) even after criterion had been reached, and the experience of a new variation of the task – Probe trials, could have resulted in overtraining. In a study conducted by Ljungberg, Apicella, and Schultz (1992), it was reported that the strong responses elicited in neurons in response (during training) to a conditioned stimulus (predicting a reward) were greatly reduced after animals were overtrained. Therefore, it is plausible that overtraining in my animals resulted in my finding of a diminished response to familiar stimuli that did not differ considerably from the response to novel cue presentations. Nevertheless, even if overtraining did not occur in my experiment, it is possible that some other aspect of the Probe trial experience might have influenced responses to familiar and novel stimuli in the rat V1.

Although the results of my electrophysiological experiments do not agree with a number of previous studies (e.g., Hager and Dringenberg, 2010; Furmanski et al., 2004), they are to some extent, consistent with the findings by Ghose, Yang, and Maunsell (2002). After monkeys were trained in an orientation discrimination task, Ghose et al., took extracellular recordings of single neurons in V1 and V2 in response to stimuli used during discrimination training. Even though animals acquired the task and improved in discrimination thresholds, learning did not result in orientation-specific response biases in V1 and V2 neuronal populations, that accounted for the orientation specificity of the behavioural improvement. It is possible that like the conclusion drawn by Ghose et al., the behavioural improvement exhibited by my rats in the altered version (from the behavioural procedure in Hager and Dringenberg, 2010) of the visual discrimination task
(addition of post-acquisition testing) was accomplished without changing the signal responses from early visual neurons.

Another possible explanation for the data obtained in my experiment could be that the level of visual discrimination task difficulty, during post-acquisition, changed the extent of V1 involvement in learning, as proposed by Ahissar and Hochstein (1997). According to Ahissar and Hochstein, tasks of greater complexity or difficulty (e.g., decreased signal discriminability or stimulus masking after variable time intervals) require increasingly specific learning processes, and therefore involve a greater recruitment of lower sensory areas where neurons are more finely tuned to orientation and position. Therefore, although my animals may have acquired a higher level of learning specificity from their experience with the more difficult Probe trials, the extracellular field recordings used in my experiment might not have captured the localised enhancement of particular neurons in V1.

With respect to the CA1 area of the hippocampus; my findings were contrary to my earlier hypothesis predicting an enhancement in visual evoked responses in CA1 to familiar versus novel stimuli. Furthermore, the results from this experiment are inconsistent with a previous report of hippocampal involvement in a visually-based, 2-pattern discrimination task (Broadbent, Squire, & Clark, 2007). Despite finding no significant differences in the acquisition rate of the task for rats with hippocampal damage when compared to intact controls, Broadbent et al., noted that only 6 out of 9 hippocampal-lesioned animals learned the task. Moreover, when a test was administered 2 weeks after training, it revealed a marked impairment in task retention among the lesioned rats in comparison to controls.
My findings are also inconsistent with those published by Tsanov and Manahan-Vaughan (2006) discussing the interaction between synaptic alterations in the visual cortex and the hippocampus. After theta-burst stimulation of the thalamocortical pathway, Tsanov and Manahan-Vaughan (2006) found a lasting enhancement of granule cell excitability in the dentate gyrus of the hippocampus that was accompanied by a concurrent V1 response potentiation. To account for the difference between my results and those reported by Tsanov and Manahan-Vaughan, it is possible that V1 response changes to visual stimuli (after discrimination training) are more readily detectable in the dentate gyrus than the CA1 of the hippocampus – where I chose to record. It is also plausible that the lack of variance in CA1 responses elicited by familiar and novel visual stimuli was a reflection of the absence of change in synaptic excitability in this part of the hippocampal formation.

Despite finding similar response amplitudes for all 3 visual stimuli, it is interesting to note that in both V1 and CA1, the visual evoked response to the novel stimulus appeared to be ever so slightly larger. From an evolutionary standpoint, an animal must be able to immediately attend and respond to unexpected stimuli to improve their chances for survival. In the past, electrophysiological recordings of the scalp, intracranial event-related potentials (ERPs), and single-neuron recordings have demonstrated that novel stimuli presentations result in a distributed activation of the prefrontal (Knight, 1984), posterior-association cortex (Yamaguchi & Knight, 1991) and temporal cortical regions including the hippocampus (Rolls, Cahusac, Feigenbaum, & Miyashita, 1993). To examine the role of the medial temporal lobe in novelty processing further, Knight (1996) measured physiological responses in humans to unfamiliar
auditory and tactile stimuli. In comparison to normal control subjects, the
electroencephalogram responses to unexpected novel stimuli in hippocampal-damaged
patients were **significantly reduced**. From his findings, Knight suggested that the
hippocampal region could be an essential component in novel stimuli detection and
response. Therefore, in my experiment, the slightly larger CA1 VEPs measured in
response to the novel stimuli could potentially be a result of novelty detection in the
hippocampus. As such, due to extensive exposure to the CS+ and CS- during
discrimination training, it is logical that these familiar images no longer elicited as strong
VEPs as the novel stimulus.

The absence of a greater response in area CA1 to familiar visual stimuli could
also be a reflection of memories becoming increasingly independent of the medial
temporal lobe after prolonged visual discrimination training (post-acquisition testing). In
a review by Wiltgen, Brown, Talton, and Silva (2003), compelling evidence was brought
forth of the involvement of specific neocortical regions in the permanent storage of
information that was *initially processed* in the hippocampus. According to these authors,
although new memories are dependent on the medial temporal lobe at the start, they
gradually become independent of this area as they are consolidated in neocortical circuits.
Hence, it is possible that responses to familiar visual stimuli in area CA1 of the
hippocampus may have been greater when animals first reached criterion, but the
enhanced response could have become diminished as discrimination training continued
(less hippocampal involvement).
5.3.2 Limitations and Future Directions

As alluded to in an earlier section of the discussion, instead of using the extracellular field recordings employed in this experiment, future studies could examine the VEP responses using a more refined electrophysiology measure, such as intracellular recordings of V1 neurons. By using this method, we will have a better opportunity to detect enhanced responses of individual neurons. Furthermore, although enhanced VEP responses to familiar stimuli were also absent in the CA1 area, a future study could investigate responses to familiar and novel stimuli in the dentate gyrus to determine if the responses could be detected in this area of the hippocampus.

Also, as it was impossible to conclude whether the lack of enhance VEPs to familiar stimuli was a result of the extra post-acquisition testing phase, it would be worthwhile to repeat the electrophysiology portion of the experiment in a group of animals immediately after task acquisition (no post-acquisition testing), and in a group after 5 days of post-acquisition testing. This would enable us to compare the VEPs (in both V1 and CA1/dentate gyrus) elicited by all stimuli in both groups to examine whether a change in VEP responses takes place after post-acquisition testing.

In addition to reporting enhanced VEPs to familiar versus novel stimuli, Hager and Dringenberg (2010) found that animals trained in the visual discrimination task exhibited higher potentiation after LTP induction in the LGN in comparison to untrained animals. From these results, Hager and Dringenberg (2010) proposed that the increase in potentiation was an indication of greater plasticity of the thalamocortical synapses following discrimination training. A natural extension of this project would be to investigate whether this greater potentiation in trained animals persists after post-
acquisition testing. Perhaps if further experience with the visual cues during post-acquisition testing changes the animal’s response to the stimuli, one can rationalise that this additional experience could potentially alter the training-induced plasticity in the visual cortex.

5.3.3 Conclusions

The results of the electrophysiology portion of this experiment are contrary to earlier hypotheses and the findings of many previous studies. In both the primary visual cortex and CA1 area of the hippocampus, the visual evoked response amplitudes elicited by familiar stimuli encountered during training were not significantly different from the measured responses to an unfamiliar novel stimulus.

5.4 General Conclusion

In Experiment I and II of this thesis, I used SC to examine the importance of ACh in attention, and memory encoding and consolidation in learning. By employing a uniquely designed post-acquisition testing procedure after visual discrimination training (composed of Regular and Probe trials), I was able to control for the peripheral side effects of SC, as well as within-subject response differences to varying doses of the drug. Through these experiments, I have provided evidence for the vital role of ACh in the traditional view of the learning and memory process, as well as in sustaining visual attention. Although I had originally set out to determine whether the cognitive deficits often attributed to learning and memory impairments by anticholinergic activity, were instead a consequence of disruptions in attention, it has become clear that ACh does not
have a unitary cognitive function. As such, it may be better to appreciate the role that
ACh plays in modulating what rats attend to, how they process and store information, and
how they use previously acquired learning strategies to guide future behaviour. Finally,
the electrophysiology portion of this thesis did not find experience-dependent changes in
V1 and CA1 responding after visual discrimination training. It is important to note that
these findings do not rule out the plastic response properties of the mature V1 and CA1,
but instead, demonstrate the complex nature of memory and synaptic encoding in the
brain.
References


Appendix A

A Typical Daily Visual Discrimination Training Sheet

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<td>L</td>
<td>L</td>
<td>R</td>
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<td>CS + ♦</td>
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<td>CS -</td>
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*Figure A.* An example of a visual discrimination training sheet used to record performance for 10 daily trials. The column furthest to the left denotes the subject number and the positive (CS+) and negative (CS-) visual stimuli that each animal discriminates between. The location of the platform within the 2 goal arms for all trials is determined by an online random sequence generator; L = Left arm, and R = Right arm. The superscript + and − signs next to the letters signify which of the two visual cues is removed during a Probe trial (+ = CS+ removed, − = CS− removed). Performance for each of the 10 trials is marked as correct or incorrect in the blank box directly below the letter indicating the platform location.
Appendix B

Table 1

Descriptive statistics and follow-up paired-samples t-test values for comparisons of performance on Regular versus Probe trials under an injection-free condition

| Performance comparison                        | Paired Differences |   |   |   |
|-----------------------------------------------|--------------------|---|--|--|---|
| Regular Trial Day 1 vs. Probe Trial Day 1     | 23.17              | 9.89 | 5 | 2.34 | .07* |
| Regular Trial Day 2 vs. Probe Trial Day 2     | 20.83              | 15.02 | 5 | 1.39 | .22 |
| Regular Trial Day 3 vs. Probe Trial Day 3     | 19.00              | 12.34 | 5 | 1.54 | .18 |
| Regular Trial Day 4 vs. Probe Trial Day 4     | 27.33              | 12.58 | 5 | 2.17 | .08* |
| Regular Trial Day 5 vs. Probe Trial Day 5     | 37.50              | 8.54  | 5 | 4.39 | .01** |

Note. M and SEM values are expressed as percent correct values. *p < .09. **p < .01.

Table 2

Descriptive statistics and follow-up paired-samples t-test values for comparisons of performance on Probe vs. Regular trials after saline and scopolamine (SC) injections

| Performance comparison                        | Paired Differences |   |   |   |
|-----------------------------------------------|--------------------|---|--|--|---|
| Probe Trial vs. Regular Trial after saline    | -15.10             | 9.21 | 11 | -1.64 | .13 |
| Probe Trial vs. Regular Trial after 0.125 SC  | -23.96             | 7.91 | 11 | -3.03 | .01* |
| Probe Trial vs. Regular Trial after 0.25 SC   | -31.77             | 10.07 | 11 | -3.15 | .01* |
| Probe Trial vs. Regular Trial after 0.5 SC    | -35.42             | 8.40  | 11 | -4.21 | .001** |
| Probe Trial vs. Regular Trial after 1.0 SC    | -36.46             | 8.21  | 11 | -4.44 | .001** |

Note. SC doses are expressed in mg/kg, and M and SEM values are expressed as percent correct values. *p = .01. **p = .001.
Appendix C

Table 1

Descriptive statistics and follow-up mixed-model t-test values for comparisons of Probe trial performance between controls and scopolamine (SC) injected animals

<table>
<thead>
<tr>
<th>Post-acquisition Day</th>
<th>Paired Differences</th>
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<td>M</td>
<td>SEM</td>
<td>df</td>
<td>t</td>
<td>p</td>
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<td>Control vs. SC rats – Day 1</td>
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<td>88</td>
<td>2.19</td>
<td>.03*</td>
</tr>
</tbody>
</table>

Note. *M and SEM values are expressed as percent correct values. *p < .05. **p < .01.